**Committee for Risk Assessment**

**RAC**

Annex 1
**Background document**
to the Opinion proposing harmonised classification and labelling at EU level of

# mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride

**EC Number: 246-147-6**
**CAS Number: 24307-26-4**

CLH-O-0000006959-53-01/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**

**18 March 2021**

# ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE

# CLH report

# Proposal for Harmonised Classification and Labelling

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

## International Chemical Identification:

# mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride

**EC Number:** **246-147-6**

**CAS Number:** **24307-26-4**

**Index Number:** **613-127-00-7**

**Contact details for dossier submitter:** **Finnish Competent Authority**
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**Finland**

**Version number: 2** **Date: 5.2.2020**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE

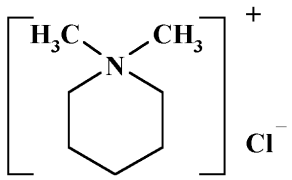
# CONTENTS

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE

# 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)** | 1,1-dimethylpiperidinium chloride |
| **Other names (usual name, trade name, abbreviation)** | |
| **ISO common name (if available and appropriate)** | mepiquat chloride |
| **EC number (if available and appropriate)** | 246-147-6 |
| **EC name (if available and appropriate)** | |
| **CAS number (if available)** | 24307-26-4 |
| **Other identity code (if available)** | 440.302 |
| **Molecular formula** | $C_7H_{16}ClN$ |
| **Structural formula** |  |
| **SMILES notation (if available)** | |
| **Molecular weight or molecular weight range** | 149.7 g/mol |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | |
| **Description of the manufacturing process and identity of the source (for UVCB substances only)** | |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | |

## 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)** |
|---|---|---|---|
| **Mepiquat chloride, CAS 24307-26-4** | **min. 99%** | Acute Tox. 4 *, H302<br>Aquatic Chronic 3, H412 | Aquatic Chronic 3, H412<br>Aquatic Chronic 4, H412<br>Acute Tox 4, H302 |

**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 |
|---|---|---|---|---|
| **N-methylpiperidine, CAS 626-67-5** | | | Flam. Liq.2 ; H225, Acute Tox 4 ; H302, Acute Tox. 4 ; H312, Skin Corr. 1B; H314, Eye Dam. 1 ; H318, Acute Tox. 3 ; H331 Aquatic Chronic 3; H412 | No |

**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 |
|---|---|---|---|---|---|
| | | | | | |

**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 |
|---|---|---|---|---|
| | | | | |

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

**Table 6:**

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors and ATE | 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 | 613-127-00-7 | 1,1-dimethylpiperidinium chloride; mepiquat chloride | 246-147-6 | 24307-26-4 | Acute Tox. 4*<br>Aquatic Chronic 3 | H302<br>H412 | GHS07<br>Wng | H302<br>H412 | - | - | - |
| Dossier submitters proposal | 613-127-00-7 | mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride | 246-147-6 | 24307-26-4 | **Modify**<br>Acute Tox. 3<br>**Add**<br>Acute Tox. 4<br>STOT-SE 2<br>Repr. 2<br>**Retain**<br>Aquatic Chronic 3 | **Modify**<br>H301<br>**Add**<br>H332<br>H371 (nervous system)<br>H361d<br>**Retain**<br>H412 | **Modify**<br>GHS06<br>Dgr<br>**Add**<br>GHS08 | **Modify**<br>H301<br>**Add**<br>H332<br>H371 (nervous system)<br>H361d<br>**Retain**<br>H412 | - | inhalation: ATE = 2.8 mg/L (dusts or mists)<br>oral: ATE = 115 mg/kg bw | - |
| Resulting Annex VI entry if agreed by RAC and COM | 613-127-00-7 | mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride | 246-147-6 | 24307-26-4 | Repr. 2<br>Acute Tox. 4<br>Acute Tox. 3<br>STOT-SE 2<br>Aquatic Chronic 3 | H361d<br>H332<br>H301<br>H371 (nervous system)<br>H412 | GHS06<br>GHS08<br>Dgr | H301<br>H332<br>H371 (nervous system)<br>H361d<br>H412 | - | inhalation: ATE = 2.8 mg/L (dusts or mists)<br>oral: ATE = 115 mg/kg bw | - |

**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** | Hazard class not assessed in this dossier | No |
| **Flammable gases (including chemically unstable gases)** | Hazard class not assessed in this dossier | No |
| **Oxidising gases** | Hazard class not assessed in this dossier | No |
| **Gases under pressure** | Hazard class not assessed in this dossier | No |
| **Flammable liquids** | Hazard class not assessed in this dossier | No |
| **Flammable solids** | Hazard class not assessed in this dossier | No |
| **Self-reactive substances** | Hazard class not assessed in this dossier | No |
| **Pyrophoric liquids** | Hazard class not assessed in this dossier | No |
| **Pyrophoric solids** | Hazard class not assessed in this dossier | No |
| **Self-heating substances** | Hazard class not assessed in this dossier | No |
| **Substances which in contact with water emit flammable gases** | Hazard class not assessed in this dossier | No |
| **Oxidising liquids** | Hazard class not assessed in this dossier | No |
| **Oxidising solids** | Hazard class not assessed in this dossier | No |
| **Organic peroxides** | Hazard class not assessed in this dossier | No |
| **Corrosive to metals** | Hazard class not assessed in this dossier | No |
| **Acute toxicity via oral route** | Harmonised classification proposed | Yes |
| **Acute toxicity via dermal route** | Data inconclusive | Yes |
| **Acute toxicity via inhalation route** | Harmonised classification proposed | Yes |
| **Skin corrosion/irritation** | Hazard class not assessed in this dossier | No |
| **Serious eye damage/eye irritation** | Hazard class no assessed in this dossier | No |
| **Respiratory sensitisation** | Hazard class not assessed in this dossier | No |
| **Skin sensitisation** | Data conclusive but not sufficient for classification | Yes |
| **Germ cell mutagenicity** | Hazard class not assessed in this dossier | No |
| **Carcinogenicity** | Data conclusive but not sufficient for classification | Yes |
| **Reproductive toxicity** | Harmonised classification proposed | Yes |
| **Specific target organ toxicity-single exposure** | Harmonised classification proposed | Yes |
| **Specific target organ toxicity-repeated exposure** | Hazard class not assessed in this dossier | No |
| **Aspiration hazard** | Hazard class not assessed in this dossier | No |
| **Hazardous to the aquatic environment** | Harmonised classification proposed | Yes |
| **Hazardous to the ozone layer** | Hazard class not assessed in this dossier | No |

## 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The hazard classification of mepiquat chloride according to Dangerous Substances Directive (DSD) 67/548/EEC was agreed in the November 1995 meeting of the Commission Working Group on the C&L of Dangerous Substances. The Group agreed to the classification as: Xn; R22-52/53.

## 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

## 5 IDENTIFIED USES

Mepiquat chloride is a plant growth regulator which is mainly used in cereals.

## 6 DATA SOURCES

The Renewal Assessment Report (2018) under Regulation (EC) 1107/2009 was used as the main data source for drafting the CLH report of mepiquat chloride. However the CLH report is an independent hazard assessment of mepiquat chloride and therefore in some cases the conclusions in the CLH report are different from those in RAR.

## 7 PHYSICOCHEMICAL PROPERTIES

### Table 8: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 101,3 kPa** | Pure: white crystalline solid (99.3 %)<br><br>Technical concentrate (TK): light yellow liquid (59.9 %) | 2001<br>dRAR B.2.3/01<br><br>1992<br>dRAR B.2.3/01 | No data on test temperature. |
| **Melting/freezing point** | Above 300°C | 2001<br>dRAR B.2.1/01 | |
| **Boiling point** | Above 320°C | 2001<br>dRAR B.2.1/02 | Decomposition begins at 320°C (indicated by smell of piperidine and total weight loss). |
| **Relative density** | $D^{20}_4 = 1.166$ | 2001<br>dRAR B.2.14/01 | |
| **Vapour pressure** | $< 10^{-8}$ Pa at 20°C and 25°C | 2001<br>dRAR B.2.2/01 | |

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE

| **Property** | **Value** | **Reference** | **Comment (e.g. measured or estimated)** |
|---|---|---|---|
| **Surface tension** | 47.4 mN/m at 20°C<br>(1 % w/w) (99.3 % pure) | 2001<br>dRAR B.2.12/01 | |
| **Water solubility** | 674.0 g/L at 20°C<br>(pH = 6.1) (99.8 % pure) | 2019<br>dRAR B.2.5/01, B.2.6/01 | Solubility was determined using OECD 105/CIPAC MT181 (equivalent to EEC Method A6). |
| **Partition coefficient n-octanol/water** | Log $P_{o/w}$ = -3.20 at 20°C<br>(pH = 4) (shake flask method)<br>Log $P_{o/w}$ = -3.55 at 20°C<br>(pH = 7) (shake flask method)<br>Log $P_{o/w}$ = -3.14 at 20°C<br>(pH = 10) (shake flask method) | 2000<br>dRAR B.2.7/01 | |
| **Flash point** | | | Solid: Not required as the melting point of the active substance is higher than 40°C.<br>Liquid: TK is an aqueous solution of non-flammable technical material, so it is implausible that vapours could be ignited and a flash point achieved. |
| **Flammability** | Not flammable | 2001<br>dRAR B.2.9/01 | A burning time of 345 secs was observed in the preliminary test. No further tests were carried out. |
| **Explosive properties** | No explosive properties | 2001<br>dRAR B.2.11/01 | The aqueous TK is not regarded as explosive as it contains only the non-explosive technical material (~60 %) and water (~40 %). |
| **Self-ignition temperature** | 311°C (relative) | 2001<br>dRAR B.2.9/02 | Autoflammability not determined for TK. |
| **Oxidising properties** | No oxidizing properties | 2001<br>dRAR B.2.13/01 | |
| **Granulometry** | - | | |
| **Stability in organic solvents and identity of relevant degradation products** | - | | |
| **Dissociation constant** | No dissociation constant | 2002<br>dRAR B.2.8/01 | Mepiquat chloride completely dissociates in aqueous solutions and therefore has no dissociation constant. |
| **Viscosity** | - | | |

## 8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

## 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

### Table 9: Summary table of toxicokinetic studies

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td><u>Absorption, distribution, elimination</u><br><br>OECD 417, 2010<br>GLP<br><br>Sprague Dawley rat<br><br>Oral (single and repeated dosing of 7 and 14 days)<br><br>1.2 and 12 mg/kg bw</td><td>Mepiquat chloride was rapidly absorbed. Bioavailability was > 78% (168 h). Urine was the major route of excretion (74 to 94% within 168 h; 52-74% within the first 12 h). Even distribution at early stages, with highest levels in liver and kidney. No evidence of biotransformation.<br><u>Summary of toxicokinetic parameters:</u>
<table>
<tr><th rowspan="2">Parameter</th><th colspan="4">Dose Group</th></tr>
<tr><th colspan="2">1.2 mg/kg bw (5 males, 5 females)</th><th colspan="2">12 mg/kg bw (5 males, 5 females)</th></tr>
<tr><th>Sex</th><th>M</th><th>F</th><th>M</th><th>F</th></tr>
<tr><td>$C_{max}$ in plasma [ppm]</td><td>0.207</td><td>0.245</td><td>2.370</td><td>2.167</td></tr>
<tr><td>$T_{max}$ in plasma [min]</td><td>40</td><td>40</td><td>60</td><td>60</td></tr>
<tr><td>$AUC_{plasma}$ [ppm equiv./h]</td><td>2.28</td><td>2.80</td><td>4.38</td><td>3.97</td></tr>
</table></td><td></td><td>dRAR B.6.1.1., 1987</td></tr>
<tr><td><u>Distribution after single oral administration</u><br>OECD 417, 2010<br>GLP<br>SPF Sprague Dawley rat<br>1.2 and 12 mg/kg bw</td><td>Mepiquat chloride was almost completely retained (83- 93%) in organs and tissues after 40 minutes. After 24 h and 48 h this amount dropped to 2% and to <1%, respectively. Highest mean equivalent concentrations were found in GIT, urinary bladder, liver and kidneys. No metabolites were detected.</td><td></td><td>dRAR B.6.1.1., 1992</td></tr>
<tr><td><u>Distribution (plasma, blood cells, bone marrow)</u><br><br>OECD 417, 2010<br>GLP<br>Crl:NMRI mice<br>2 oral doses, 500 mg/kg bw at interval of 24 h<br>Radioactive residues measured 1 h after second dosing</td><td><u>Mean total of radioactive residues</u><br>plasma: 11.09 µg Eq/g<br>blood cells: 5.69 µg Eq/g<br>bone marrow: 64.62 µg Eq/g</td><td>Additional study on exposure of bone marrow for assessment of mouse micronucleus assay (Table 51).</td><td>dRAR B.6.4.2., 2017</td></tr>
<tr><td>In vitro comparative metabolism in mouse (CD-1) rat (Sprague-Dawley), dog (beagle) and human</td><td>Trace evidence of a hydroxylated metabolite at the highest concentration in the rat and the dog. No metabolites unique to human hepatocytes observed.</td><td></td><td>dRAR B.6.1.1., 2015</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>hepatocytes<br>Preliminary phase (rat): 0,1,3,10,30 and 100 µM<br>Interspecies comparison phase:<br>10 and 100 µM</td><td></td><td></td><td></td></tr>
<tr><td>Dermal (single dosing)<br>OECD 427<br>GLP<br><br>4 male rats</td><td>
<table>
<tr><th>Parameter</th><th colspan="4">Dose Group</th></tr>
<tr><td>Nominal dose</td><td colspan="2">0.038 mg/cm$^2$</td><td colspan="2">3 mg/cm$^2$</td></tr>
<tr><td>Actual dose (mg/cm$^2$)</td><td>0.036</td><td>0.038</td><td>2.89</td><td>3.05</td></tr>
<tr><td>Sacrifice time (h)</td><td>10</td><td>96</td><td>10</td><td>96</td></tr>
<tr><td>Material absorbed</td><td>1.47%</td><td>2.59%</td><td>1.75%</td><td>0.95%</td></tr>
</table>
</td><td></td><td>dRAR B.6.1.1., 2003</td></tr>
</table>

Table 10: Summary of the distribution in organs and tissues. Mean equivalent concentrations of radiolabel after oral administration. (Ref. dRAR B 6.1.1., 1992).

| Organ / Tissue | 1.2 mg/kg bw | | | | 12 mg/kg bw | | | |
|---|---|---|---|---|---|---|---|---|
| | 40 min p. a. | | 48 h p. a. | | 40 min p. a. | | 48 h p. a. | |
| | male | female | male | female | male | female | male | female |
| liver | 3.4664 | 1.5671 | 0.0032 | 0.0012 | 21.5520 | 14.2339 | 0.0379 | 0.0392 |
| kidney | 5.8138 | 1.8191 | 0.0044 | 0.0019 | 26.2244 | 13.8332 | 0.0938 | 0.0537 |
| fat | 2.692 | 0.0509 | 0.0001 | n.d. | 0.9686 | 0.6876 | 0.0067 | 0.0045 |
| spleen | 0.3106 | 0.1255 | 0.0008 | 0.0005 | 1.0192 | 1.3582 | 0.0095 | 0.0107 |
| heart | 1.6994 | 0.5324 | 0.0009 | 0.0028 | 3.0073 | 6.2291 | 0.0124 | 0.0195 |
| bone | 0.343 | 0.0610 | 0.0013 | 0.0031 | 0.3337 | 0.4229 | 0.0152 | 0.0165 |
| bone marrow | 0.4266 | 0.1559 | 0.0036 | 0.0029 | 1.5223 | 5.0215 | 0.0383 | 0.0217 |
| lung | 1.7188 | 0.9338 | 0.0009 | 0.0005 | 2.9691 | 3.5480 | 0.0134 | 0.0151 |
| brain | 0.0640 | 0.0159 | n.d. | n.d. | 0.1114 | 0.1390 | 0.0019 | 0.0019 |
| muscle | 0.1655 | 0.0655 | 0.0172 | 0.0131 | 0.4880 | 0.4934 | 0.2209 | 0.2116 |
| testes | 0.1621 | --- | 0.0095 | --- | 0.4538 | --- | 0.0801 | --- |
| ovaries | --- | 0.4509 | --- | 0.0006 | --- | 4.0492 | --- | 0.0173 |
| uterus | --- | 0.1235 | --- | 0.0002 | --- | 1.9897 | --- | 0.0112 |
| pituitary gland | 1.5195 | 0.2170 | 0.0363 | 0.0075 | 5.0711 | 2.7044 | 0.1004 | 0.0790 |
| thyroid | 2.6037 | 0.7530 | 0.0008 | 0.0006 | 4.5346 | 4.1942 | 0.0161 | 0.0336 |
| adrenals | 1.1816 | 0.6094 | 0.0017 | 0.0009 | 5.4170 | 4.6953 | 0.0603 | 0.0414 |
| pancreas | 0.4138 | 0.1614 | 0.0008 | 0.0004 | 1.6008 | 1.6227 | 0.0091 | 0.0106 |
| urinary bladder | 78.3556 | 4.4388 | 0.0339 | 0.0043 | 228.1660 | 30.3894 | 0.5209 | 0.7211 |
| GIT | 5.6067 | 8.1535 | 0.0027 | 0.0179 | 98.7995 | 100.852 | 0.0217 | 0.0990 |
| carcass | 0.6646 | 0.1944 | 0.0135 | 0.0092 | 0.8022 | 0.9719 | 0.1701 | 0.1725 |

| **Organ / Tissue** | **1.2 mg/kg bw** | | | | **12 mg/kg bw** | | | |
|---|---|---|---|---|---|---|---|---|
| | **40 min p. a.** | | **48 h p. a.** | | **40 min p. a.** | | **48 h p. a.** | |
| | **male** | **female** | **male** | **female** | **male** | **female** | **male** | **female** |
| liver | 3.4664 | 1.5671 | 0.0032 | 0.0012 | 21.5520 | 14.2339 | 0.0379 | 0.0392 |
| plasma | 0.3330 | 0.1729 | 0.0001 | n.d. | 1.7044 | 1.8710 | 0.0028 | 0.0032 |
| blood | 0.2516 | 0.1206 | 0.0004 | 0.0004 | 1.1649 | 1.3534 | 0.0030 | 0.0036 |

# 10 EVALUATION OF HEALTH HAZARDS

## Acute toxicity

### 10.1 Acute toxicity - oral route

**Table 11: Summary table of animal studies on acute oral toxicity with mepiquat chloride**

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| OECD TG 401 (1987)<br><br>Oral, rat<br><br>GLP | Wistar rat<br><br>5/sex/group | Mepiquat chloride<br>(purity 57.9 %) | 100, 200, 464, 1470, 2150 mg/kg bw in water<br><br>single oral dose by gavage | Males:<br>464 mg/kg bw corresponding to active ingredient dose (a.i.) 270 mg/kg bw<br><br>Females:<br>200 - 464 mg/kg bw corresponding to active ingredient doses (a.i.) 115 - 270 mg/kg bw | dRAR B.6.2.1., 1989<br><br>**Key study** |
| OECD TG 401 (1987)<br><br>Oral, mouse<br><br>GLP | NMRI mouse<br><br>5/sex/group | Mepiquat chloride<br>(purity 57.9 %) | 100, 200, 464, 1470, 2150 mg/kg bw in water<br><br>single oral dose by gavage | Both sexes:<br><br>780 mg/kg bw corresponding to active ingredient dose (a.i.) 450 mg/kg bw | dRAR B.6.2.1., 1989 |

Specific human information on acute toxicity of mepiquat chloride is not available.

Table 12: Summary table of other studies relevant for acute oral toxicity

| Type of study/data | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Observations | Reference |
|---|---|---|---|---|---|
| Acute effects in pre-weaning Wistar rats (oral administration)<br><br>GLP | Wistar pre-weaning rats<br><br>35-37 males + 36-41 females in dose groups 30, 60 and 120 mg a.i./kg bw/day and 6 males and 10 females in dose group 200 mg a.i./kg bw/day | Mepiquat chloride<br>(purity 56.7 %) | 30, 60, 120, 200 mg a.i./kg bw/day in water<br><br>from day 11 p.p. to day 21 p.p. | Both sexes:<br>Increased mortality in the dose groups 120 and 200 mg a.i./kg bw/day | dRAR<br>B.6.7.1., 2006 |

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

Wistar rat

In the oral toxicity study (dRAR B.6.2.1, 1989) Wistar rats (5/sex/group) were administered by gavage a single oral dose of mepiquat chloride (purity: 57.9 % with 44.3 % water) dissolved in distilled water at dose levels of 100, 200, 464, 1470 and 2150 mg/kg bw (1.000 to 21.500 g/100 ml; administration volume: 10 ml/kg). In a pretest with 2 male and 2 female animals the doses 2000 and 200 mg/kg bw were tested. 2000 mg/kg bw caused 100% mortality but 200 mg/kg bw was not lethal. Because 50% mortality occurred in the 464 mg/kg bw dose group in the main study, two additional doses were tested (200 and 100 mg/kg bw).

Observation period was 14 days for doses 100 to 464 mg/kg bw, 0 days for 1470 and 2150 mg/kg bw. Signs and symptoms were recorded several times on the day of administration, and at least once each workday. Necropsies, with gross-pathological examination, were performed on fasted animals.

Mortalities occurred at dose levels of ≥ 464 mg/kg bw within 1 – 24 h after dosing. Clinical signs including poor general state, dyspnea, apathy, abdominal position, staggering, twitching, compulsary gnawing and cyanosis were observed up to 1 hour after administration at dose levels of > 464 mg/kg bw in both sexes. No signs of toxicity were observed at dose levels of ≤ 200 mg/kg bw. Mean body weight gains were not significantly affected by the treatment. Gross examination at necropsy revealed general congestion in animals which died but no abnormal observations were made in survivors killed at scheduled termination. The acute oral $LD_{50}$ of mepiquat chloride in the rat was approximately 464 mg/kg bw (equivalent to 270 mg a.i./kg bw) for males and 200 – 464 mg/kg bw (equivalent to 115-270 mg a.i./kg bw) in females.

NMRI mouse

In the oral toxicity study (dRAR B.6.2.1, 1989) groups of fasted NMRI mice (5/sex/group) were administered by gavage a single oral dose of mepiquat chloride (mepiquat chloride 600 g/l) in distilled water at dose levels of 100, 200, 464, 1470 and 2150 mg/kg bw (1.00 – 21.500 g/100 ml, administration volume: 10 ml/kg). Observation period was 14 days. Signs and symptoms were recorded several times on the day of administration, and at least once each workday. Necropsies, with gross-pathological examination, were performed on fasted animals.

Mortalities occurred at dose levels of ≥ 464 mg/kg bw within 1h after dosing. Clinical signs in both sexes included signs of poor general state, dyspnoea, apathy, abdominal position, lateral position, staggering, twitching, clonic convulsions, exsiccosis. Additionally tremor, piloerection and weight reduction were observed in females in the 2150 mg/kg bw dose group. Most of the symptoms were reversible within 4 hours except for weight reduction which was observed until day 13 in survivors. Gross examination at necropsy revealed general congestion in animals which died but no pathological findings were noted in survivors killed at scheduled termination. According to study report the acute oral $LD_{50}$ of mepiquat chloride in mice was approximately 780 mg/kg bw corresponding to active ingredient dose (a.i.) 450 mg/kg bw for both sexes.

Preweaning Wistar rat

Acute effects in pre-weaning Wistar rats (dRAR B.6.7.1, 2006) support the previous results. The test substance solutions of mepiquat chloride (purity 56.7%) were administered to the pups by gavage days 11-21 p.p. at dose levels of 0 (doubly distilled water), 30, 60, 120 and 200 mg a.i./kg bw/day in a volume of 10 ml/kg bw. The number of pups dosed were (males/females) 40/32, 35/36, 35/39, 37/41 and 6/10 for dose levels of 0, 30, 60, 120 and 200 mg/kg bw/day, respectively. Mortality of pups after administration was observed in all dose groups except the low dose group (30 mg a.i./kg bw). The acute lethality of offspring was 4%, 55% and 100% at 60, 120 and 200 mg a.i./kg bw/day, respectively. Deaths occurred approximately 2-4 hours after the direct dose to the pups on days 11-16 p.p. Tremors and lateral position were observed in pups of 120 mg a.i./kg bw/day group.

### 10.1.2 Comparison with the CLP criteria

The acute oral $LD_{50}$ of mepiquat chloride in the rat was approximately 464 mg/kg bw for males and 200 – 464 mg/kg bw in females. Reference dose levels have been corrected in the remainder of the toxicological assessment and are expressed in terms of mepiquat chloride as appropriate. The values obtained relate to test substance, which contains 57.9% w/w mepiquat chloride and 44.3% water. Considering the content of a.i., the $LD_{50}$ value for females is 115 - 270 mg a.i./kg bw. Based on the results of this study, females are more sensitive and the classification should be based on the $LD_{50}$ for females.

The acute oral $LD_{50}$ of mepiquat chloride in mice was approximately 450 mg a.i./kg bw for both sexes, as it was mentioned in the study report that the values obtained relate to test substance, which contains 57.9% w/w mepiquat chloride (and 44.3% water).

Classification for acute oral toxicity under Regulation (EC) No 1272/2008 (Section 3.1) is required to category 3 for substances with an acute oral $LD_{50}$ value (or estimated LD50 value) of $50 < ATE \leq 300$ mg/kg bw. The lowest acute oral $LD_{50}$ was 115 mg a.i./kg bw.

### 10.1.3 Conclusion on classification and labelling for acute oral toxicity

As the $LD_{50}$ of 115 mg a.i./kg bw lies within the criteria for classification as Acute Tox. 3, the minimum classification as Acute Tox. 4 is proposed to be modified to Acute Tox 3, H301 – Toxic if swallowed, with an ATE of 115 mg a.i./kg bw.

## 10.2 Acute toxicity - dermal route

**Table 13: 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 TG 402 (1987)<br>GLP | Wistar rat<br>5 males + 5 females | Mepiquat chloride<br>(57.9 %) | 2000 mg/kg bw in water, corresponding to active ingredient dose (a.i.) 1160 mg/kg bw<br>Exposure: 24 hours (semi-occlusive) | Both sexes:<br>> 1160 a.i. mg/kg bw | dRAR B.6.2.2., 1989 |

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

A dose of 2000 mg/kg bw (equivalent to >1160 mg a.i./kg bw) mepiquat chloride (purity: 57.9 % with 44.3% water) was applied undiluted under a semi-occlusive dressing to the clipped dorsal and dorsolateral skin of five male and five female Wistar rats for 24 h (dRAR B.6.2.2, 1989). After removal of the dressing the application site was rinsed with water. Mortality and signs of toxicity were recorded during the 14-day observation period. Scoring of the treated skin for dermal reactions was performed at 30 - 60 minutes after removal of the dressing and at 7 and 14 days after treatment. Necropsy with gross pathological examination was conducted on fasted animals.

There were no deaths. Clinical signs of toxicity or local reaction to treatment were not observed. However, there was a transient reduction in mean body weight at day 7 on females but the mean body weight increased until the end of the study. No pathological findings were noted. The acute dermal $LD_{50}$ of mepiquat chloride in the rat was >1160 mg a.i./kg bw for both sexes.

### 10.2.2 Comparison with the CLP criteria

Under the conditions of the study, the acute dermal $LD_{50}$ of the test substance in rats of both sexes was > 2000 mg /kg bw. It was mentioned in the study report that the values obtained relate to test substance, which contains 57.9% w/w mepiquat chloride (and 44.3% water). Considering the content of a.i., the $LD_{50}$ value for rat is >1160 mg a.i./kg bw. The classification should be based on this $LD_{50}$ value, however this result leaves open what would the mortality be on doses between 1160 - 2000 mg a.i./kg bw.

### 10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Data is inconclusive for dermal toxicity classification according CLP Regulation (EC) No. 1272/2008, because doses between 1160 -2000 mg a.i./kg bw are not tested.

## 10.3 Acute toxicity - inhalation route

**Table 14: 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 |
|---|---|---|---|---|---|
| OECD TG 403 (2009)<br>GLP | Wistar (SPF Wistar/Chbb) rat<br>5 males + 5 females | Mepiquat chloride<br>(purity %: not stated)<br>liquid (water) aerosol<br>Particle size: 2.7 µm - 2.9 µm | 2.59 and 4.89 mg/L<br>Exposure: 4 hours | Males:<br>>4.89 mg/L, equivalent to >2.84 mg a.i./L<br>Females:<br>≥4.89 mg/L, equivalent to ≥ 2.84 mg a.i./L | dRAR B.6.2.3., 1991 |

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

Groups of 5 male and 5 female Wistar (SPF Wistar/Chbb) rats were exposed (head-nose) for four hours to an aerosol of mepiquat chloride (purity %: not stated) (dRAR B.6.2.3, 1991). Vehicle or negative control groups were not tested. The mean analytical concentrations were 2.59 or 4.89 mg/L. The test substance was a solution. For technical reasons 50 weight % of the test substance in water was used. Sampling frequency was one sample per concentration group about hourly. Particle size was analysed by the impactor method and one sample was taken per test group for analysis. This deviated from the OECD guideline where particle size distribution should be determined at least twice each 4 hour exposure.

All animals were observed for signs of ill health or reaction to treatment during and after treatment for 14 days following the exposure. Body weights were recorded before treatment and on 7 and 14 days after exposure. Body weight was measured less often than what recommended in the guideline and individual weight data or individual records of symptoms were not given in the study report. All animals were grossly necropsied after scheduled termination on day 14 or premature death.

The concentration measurements showed mean analytical concentration of 2.59 mg/L ± 0.213 (nominal concentration 17.23 mg/L) and 4.89 ± 0.586 (nominal concentration 119.3 mg/L). The mass median aerodynamic diameter 50 % was 2.9 µm (geometrical standard deviation 4.0) and 2.7 µm (geometrical standard deviation 4.5) respectively for the 2.59 and 4.89 mg/L doses. For both doses it was reported that a respirable dust aerosol fraction that might reach the alveolar region was 81% (particles with an aerodynamic diameter of 5.5 µm).

One male rat and 2 female rats of the 4.89 mg/L dose died within 24 h after dosing. Clinical findings observed during the exposure period of low dose group were irregular, accelerated and intermittent respiration. Eyelid closure was observed in all animals. In the high dose group irregular, accelerated, intermittent and gasping respiration and eyelid closure were observed. After exposure and during the observation period ruffled fur was observed in all animals of the low dose group in addition accelerated and intermittent respiration were observed in some animals. After 48 hours all animals were without findings. In the high dose group, accelerated respiration was seen until day 5. Other signs of toxicity were intermittent respiration, respiratory sounds, abdominal, lateral or squatting position in individual animals, tonic- clonic convulsions and discoloured fur with test substance and ruffled fur (all survivors). After day 6 all survivors were without findings.

### 10.3.2 Comparison with the CLP criteria

Under the conditions of the study, the acute inhalation (4h) $LC_{50}$ for mepiquat chloride as liquid aerosol in rats was determined to be ≥4.89 mg/L. Based on the information from the study report there was uncertainty regarding the purity, however, it was reported in the DAR that the purity was 58 %. Considering a purity of 58 % the $LC_{50}$ for mepiquat chloride is ≥2.84 mg a.i./L for both sexes (> 2.84 mg active ingredient/L for males and approx. 2.84 mg active ingredient/L for females). The case for classification is borderline as mortality was seen in 1/5 in males and 2/5 in females. The highest tested concentration seemed to be too low. However, mortalities support the conclusion that in the dose of 5 mg/L mortality of >50% could be expected. Higher concentration was not tested, but the results of lethality and clinical examination were considered sufficient to characterize the toxic potential of the substance by inhalation.

### 10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No precise $LC_{50}$ was derived, but based on the results it was concluded that the LC50 in the females, which was the most sensitive sex, lies between 2.8 and 5 mg a.i./L. This range coincides with the numeric criteria of $1.0 < LC_{50} \leq 5.0$ mg/L (dusts and mists) for classification as Acute Tox 4, H332 - Harmful if inhaled. Since no precise $LC_{50}$ is available the DS proposes to use the default ATE of 2.8 mg a.i./L for dusts and mists classified in category 4 for acute inhalation toxicity.

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

***Acute oral toxicity***

The dossier submitter (DS) proposed Acute Tox. 3; H301 with an ATE of 115 mg/kg bw based on an acute oral toxicity study in rats where ca. 50% mortality (males 2/5, females 3/5) occurred at 269 mg/kg bw and no mortality at the next lower dose of 115 mg/kg bw.

***Acute dermal toxicity***

No mortality was observed in a rat acute dermal toxicity study at a test substance dose of 2000 mg/kg bw. The test substance was an aqueous solution of mepiquat chloride and the dose was not adjusted for mepiquat chloride content of 58%; thus, the actual dose of mepiquat chloride was 1160 mg/kg bw. The DS proposed no classification based on inconclusive data as doses between 1160 and 2000 mg/kg bw have not been tested.

***Acute inhalation toxicity***

The DS proposed Acute Tox. 4; H332 with an ATE of 2.8 mg/l based on an acute inhalation toxicity study in rats where several animals (males 1/5, females 2/5) died at the highest tested concentration of 2.8 mg/l.

### Comments received during consultation

2 MSCAs supported the DS's proposal.

### Assessment and comparison with the classification criteria

***Acute oral toxicity***

The current classification is Acute Tox. 4*; H302. The DS presented two standard acute oral toxicity studies, one in rats and one in mice. To provide a more complete picture, additional studies reporting mortalities are included in the following table. The two studies highlighted in grey are described in more detail thereafter.

| **Overview of rat and mouse oral studies with mepiquat chloride reporting mortalities** | | |
|---|---|---|
| **Study** | **Mortality, $LD_{50}$** | **Remarks** |
| ***Rat*** | | |
| Acute oral toxicity, Wistar rat (1989) | $LD_{50}$ ca. 270 mg/kg bw | Fasted |
| Acute oral (gavage) neurotoxicity, Wistar rat (2002) | 1/20 at 700 mg/kg bw | Probably non-fasted |
| PNDT oral (gavage) range-finding, Wistar rat (1992) | 5/10 at 600 mg/kg bw after 1-2 doses | Non-fasted, pregnant |
| DNT oral (gavage) range-finding, Wistar rat (2006) | Dams: mortality from 200 mg/kg bw/d (no further details available)<br>Pups: increased mortality from 75 mg/kg bw/d after the start of dosing on PND 11 | Dams dosed GD 6 to LD 10, pups dosed PND 11-21 |
| DNT oral (gavage), Wistar rat (2006) | Dams: no mortality at 60 mg/kg bw/d<br>Pups: increased mortality at 60 mg/kg bw/d after the start of dosing on PND 11 | Dams dosed GD 6 to LD 10, pups dosed PND 11-21 |
| Acute effects in pre-weaning Wistar rats, oral (gavage) (2006) | Pups, 120 mg/kg bw/d: 44% mortality after a single dose (PND 11), 51% mortality PND 11-13 | Dams did not receive the test substance, pups dosed PND 11-21 |
| ***Mouse*** | | |
| Acute oral toxicity, NMRI mouse (1989) | $LD_{50}$ 450 mg/kg bw | Fasted |
| Micronucleus test, oral (gavage), NMRI mouse (2002) | 2/5 after a single dose of 630 mg/kg bw | Probably non-fasted |

PNDT = prenatal developmental toxicity; DNT = developmental neurotoxicity

Acute oral toxicity study in rats (1989)

The test was conducted according to OECD TG 401 and under GLP. The test substance was an aqueous formulation reported to contain 57.9% w/w mepiquat chloride and 44.3% water. The doses in the study report relate to this aqueous formulation and have to be multiplied by 0.579 to obtain the doses of mepiquat chloride. The study employed 5 young adult animals per sex and group and the observation period was 14 days. Mortality rates at the individual dose levels are provided in the table below. The animals died 1 hour to 1 day after test substance administration.

| Mortality in the rat acute oral toxicity study (1989) | | | |
|---|---|---|---|
| **Dose (mg/kg bw)** | | **Mortality** | |
| **Test substance** | **Mepiquat chloride** | **Male** | **Female** |
| 100 | 58 | 0/5 | 0/5 |
| 200 | 116 | 0/5 | 0/5 |
| 464 | 269 | 2/5 | 3/5 |
| 1470 | 851 | 5/5 | 5/5 |
| 2150 | 1240 | 5/5 | 5/5 |

The combined $LD_{50}$ for rats was estimated by the study authors to be about 464 mg/kg bw, that is ca. 270 mg/kg bw as mepiquat chloride. No calculated value was available, presumably because the conditions for fitting the probit model were not considered met. No mortality or clinical signs of toxicity were observed at 116 mg/kg bw as mepiquat chloride, and RAC does not support the DS's proposal to choose 115 mg/kg bw as an $LD_{50}$ for this study.

Study on acute effects in pre-weaning rats (2006)

This non-standard study was a follow-up study to a developmental neurotoxicity (DNT) study (2006). The DNT study reported increased mortality of pups after the start of direct gavage dosing on PND 11 at the top dose of 60 mg/kg bw/d. The aim of the follow-up study was to determine if the mortality in the DNT study was a consequence of the acute toxicity of the test compound or a developmental effect. For this purpose only the offspring was directly exposed to the test compound without previous dosing of the dams. 10 litters per dose level were used at 0, 30, 60 and 120 mg/kg bw/d, while only 2 litters were exposed to 200 mg/kg bw/d.

Pup mortality within the first 3 days of dosing (PND 11-13) was ca. 50% at 120 mg/kg bw/d and 100% at 200 mg/kg bw/d compared to 1% in the control (see the table below). The pups died 2-6 hours after dosing.

| Study on acute effects in pre-weaning rats (2006), pups dosed via gavage from PND 11 | | | | |
|---|---|---|---|---|
| **Dose (mg/kg bw/d)** | **No. of litters** | **Number of live pups (% mortality)** | | |
| | | **PND 11 before dosing** | **PND 11 after dosing** | **PND 13** |
| **0** | 9 | 72 | 72 (0%) | 71 (1%) |
| **30** | 10 | 71 | 71 (0%) | 69 (3%) |
| **60** | 10 | 74 | 73 (1%) | 71 (4%) |
| **120** | 10 | 78 | 44 (44%) | 38 (51%) |

| | | | | |
|---|---|---|---|---|
| **200** | 2 | 16 | 0 (100%) | 0 (100%) |

Conclusion

The lowest $LD_{50}$ value from a standard study with adult animals is about 270 mg/kg bw (acute oral toxicity study in rats, 1989). An acute study in pre-weaning rats (2006) indicates that juvenile animals are more sensitive, with an $LD_{50}$ around 120 mg/kg bw. RAC notes that 11-day old pups are likely to be generally more sensitive than adults to many substances (e.g. due to incompletely developed metabolism and elimination mechanisms).

Although the acute toxicity study in rat pups provides a lower $LD_{50}$ than the standard study in adults, the Guidance on the application of the CLP criteria (CLP guidance) states that *"standard acute toxicity studies should be the primary source of information for acute toxicity classification"*. Only when such data are not available, information from studies conducted for other endpoints can be used (CLP guidance, 3.1.3.3.5). The OECD test guidelines (TG) for acute oral toxicity testing (OECD TG 420, 423, 425) clearly specify that young adult animals, approximately 8-12 weeks old, should be used.

During the RAC discussion the following statement from the CLP guidance (3.1.2.3.2) was mentioned: *"If there is a wide range of ATE values from the same species, it may be informative to consider the studies collectively, to understand possible reasons for the different results obtained. This would include consideration of factors such as the sex and age of the animals…"*. The reference to "age" in that passage is understood by RAC as a recommendation to check whether the age of the animals was in line with the OECD TGs.

Given that using data from the juvenile rats (2006) would be inconsistent with the very clear guidance on the age of rats suitable for acute testing (see above), RAC agrees with the DS to base the classification on the standard acute study in adult rats (1989). However, RAC considers 270 mg/kg bw to be the appropriate $LD_{50}$ from this study, rather than the dose of 115 mg/kg bw proposed by the DS.

In conclusion, RAC proposes classification as **Acute Tox. 3; H301** with an **ATE** of **270 mg/kg bw** based on an acute toxicity study in adult rats.

***Acute dermal toxicity***

One acute dermal toxicity study is available. It was conducted in 1989 according to OECD TG 402 and under GLP. The test substance was the same as in the oral studies, i.e. an aqueous solution containing 57.9% mepiquat chloride. This substance was applied to 5 Wistar rats per sex for 24 hours under semi-occlusive dressing at a limit dose of 2000 mg/kg bw, corresponding to 1160 mg/kg bw mepiquat chloride. The observation period was 14 days. No mortality or clinical signs of toxicity were observed. However, the dose of mepiquat chloride was below the prescribed limit dose of 2000 mg/kg bw.

It is not known whether mortality would occur in a dermal study at 2000 mg/kg bw mepiquat chloride. Mortality of adult rats in oral studies started above 100 mg/kg bw. Oral absorption after a gavage application is above 70% (single application of 12 mg/kg bw; a toxicokinetic study in Sprague-Dawley rats from 1987) while dermal absorption is about 2% (10-hour application of a 30% solution; an *in vivo* study in rats from 2003). Taking into account the oral $LD_{50}$ and the data on oral and dermal absorption, it is likely that the dermal $LD_{50}$ in rats is above 2000 mg/kg bw mepiquat chloride. Still, some uncertainty

about possible mortality at 2000 mg/kg bw mepiquat chloride remains. Therefore, RAC agrees with the DS's proposal of **no classification due to inconclusive data**.

***Acute inhalation toxicity***

One acute inhalation study is available. It was conducted in 1991 according to OECD TG 403 and under GLP. 5 Wistar rats per sex and concentration were exposed for 4 hours to a liquid aerosol of the test substance at measured concentrations of 2.6 and 4.9 mg/l. Purity of the test substance is not stated in the study report, but it was a liquid and the DS assumes (based on the information in the DAR) that the purity was the same as in the oral and dermal studies, i.e. 58%. The corresponding mepiquat chloride concentrations are then 1.5 mg/l and 2.8 mg/l. The MMAD was around 2.8 µm and the observation period was 14 days.

1 male and 2 females died at 2.8 mg/l mepiquat chloride on the day of exposure, no mortality was observed at the lower concentration. Clinical signs included accelerated respiration and tonic-clonic convulsions. As the female mortality at the higher concentration was 40%, the female $LC_{50}$ lies close to 2.8 mg/l. This concentration corresponds to Category 4 (1 mg/l < ATE ≤ 5 mg/l). RAC agrees with the DS's proposal of **Acute Tox. 4; H332** with and **ATE** of **2.8 mg/l (dusts or mists)**.

## 10.4 Skin corrosion/irritation

Not assessed in this dossier.

## 10.5 Serious eye damage/eye irritation

Not assessed in this dossier.

## 10.6 Respiratory sensitisation

Not assessed in this dossier.

## 10.7 Skin sensitisation

**Table 15: 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 |
|---|---|---|---|---|---|
| Local Lymph Node Assay (LLNA)<br>OECD TG 429 (2010)<br>GLP | Mice, CBA/ CaOlaHsd mice<br>5 females | Mepiquat chloride<br>purity 98.1 % (w/w) | Induction topical: 10 %, 25 % and 50 % w/w in ethanol/water (3+7 v/v)<br>IV injection: 20 µCi of $^{3}$H-methyl thymidine.<br>Vehicle controls: ethanol/water (3+7 v/v) and acetone/olive oil (4+1 v/v) | The proliferation index values did not increase 3 times over the control values and were 1.1, 1.2 and 1.2 at treatment concentrations of 10 %, 20 % and 50 %, respectively. As a conclusion, no evidence of a potential for | dRAR B.6.2.6 (2019)<br>**(key study)** |

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Results | Reference |
|---|---|---|---|---|---|
| | | | Positive control: 25 % w/w mixture of HCA in acetone/olive oil (4+1 v/v) | sensitization of mepiquat chloride was seen in this assay. | |
| Report on the study of the sensitizing effect of mepiquat chloride in guinea pigs according to "Proposed Rules" of EPA<br>non-GLP | Pirbright White guinea pigs<br>12 males<br>Preliminary test: 5/group | Mepiquat chloride<br>(purity not stated) | The preliminary test: 100 µl of the test substance was applied intracutaneously at concentrations of 10 %, 25 % and 50 % w/w.<br>Intracutaneous induction and challenge phases with a 10 % mepiquat chloride. | The dose level selected for intracutaneous induction and challenge phases were too high as necrotic skin changes were observed. The study is considered not acceptable and does not seem suitable to study skin sensitization of mepiquat chloride. | dRAR B.6.2.6 (1978) |

Specific human information on skin sensitising properties of mepiquat chloride is not available.

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

Local Lymph Node Assay (LLNA)

The potential of the mepiquat chloride to cause skin sensitation was evaluated in a Local Lymph Node Assay (LLNA) (dRAR B.6.2.6, 2019). The study was conducted according to the principles of GLP and was mainly performed according to OECD guideline 429 (2010). However, there were some minor deviations. At the start of the study, animals should be between 8-12 weeks old, but 12-13 weeks old were used. The relative humidity in the animal room was between 13-45 % instead of 45-65 % for few hours. The vehicle samples and reserve samples drawn for a possible future formulation analysis were 5 mL aliquots instead of 1 mL aliquots. These deviations do not most likely affect the validity of the study.

The highest test item concentration, which could be technically used, was a 50 % solution in ethanol/water (3+7 v/v). To determine the highest non-irritant test concentration, a pre-test was performed. Two mice were treated by topical application to the dorsal surface of each ear with test item concentrations of 25 % and 50 % once daily each on three consecutive days. At the tested concentrations the animals did not show any signs of systemic toxicity. Both animals showed a very slight erythema of the ear skin (score 1).

Three concentrations of the test substance (10 %, 25 % and 50 %) in ethanol:water (3+7 v/v) were selected and topically applied to female mice (5 mice/group). The application was spread over the entire dorsal surface of each ear once daily for three consecutive days. Two vehicle control groups and a positive control group were maintained under the same environmental conditions and treated in the same manner as the test animals. The vehicle control animals were treated with the vehicle for the test item (ethanol/water, 3+7 v/v) or the vehicle for the positive control item only (acetone:olive oil, 4+1 v/v)) or with the positive control (a 25 % w/v mixture of alpha-hexyl cinnamaldehyde (HCA) in acetone:olive oil (4+1 v/v).

On day 6, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine and the obtained values were used to calculate proliferation indices. No mortality or any signs of systemic toxicity were observed during the study.

Stimulation Indices (S.I.) of 1.1, 1.2, and 1.2 were determined with the test item at concentrations of 10 %, 25 %, and 50 % in ethanol/water (3+7 v/v), respectively (Table 16). The EC3 value could not be calculated, since the proliferation index values did not increase 3 times over the control values. The mean S.I. determined for the concurrent positive control was 7.6.

Table 16: Skin sensitisation potential of mepiquat chloride in LLNA test (B.6.2.6-8.)

| **Group** | **no/group** | **Mean dpm[(a)]** | **Std. Dev** | **SI[(b)]** | **SI > 3** |
|---|---|---|---|---|---|
| Negative control | 5 | 1565.7 | 517.0 | 1.0 | No |
| 10 % mepiquat chloride | 5 | 1659.3 | 841.3 | 1.1 | No |
| 25 % mepiquat chloride | 5 | 1906.9 | 995.8 | 1.2 | No |
| 50 % mepiquat chloride | 5 | 1824.7 | 145.2 | 1.2 | No |
| Vehicle for positive control | 5 | 1790.3 | 699.4 | 1.0 | No |
| Positive (concurrent) control | 5 | 13667.9 | 2264.1 | 7.6* | Yes |

*: Statistically significant vs. concurrent control
(a): Mean dpm/animal; sum of measured values from lymph nodes of all animals within a group divided by the number of animals in that group (5 animals)
(b): Stimulation index relative to the mean of the negative control group (Group 1)

Furthermore, a statistically significant increase in ear weights was observed in the high dose group in comparison to the vehicle control group ($p<0.05$). However, this was not biologically relevant, as the observed increase did not exceed the threshold value of 25 % for excessive local skin irritation mentioned in OECD guideline 429. Furthermore, the cut-off value (1.1) of the ear weight index for a positive response regarding ear skin irritation reported for BALB/c mice was not reached or exceeded in any of the treated groups. No statistically significant or biologically relevant increase in lymph node weight or lymph node cell count was observed in any of the test item treated groups. All calculations were performed with a validated test script of "*R*" (Table 17).

Table 17: Ear weight, lymph node weight and lymphocyte cell count (B.6.2.6-7.)

| **Group** | **Ear weight (mg)** | **Lymph node weight (mg)** | **Lymphocyte cell count ($\times 10^6$ per animal)** |
|---|---|---|---|
| Vehicle (ethanol/water) | 24.85 ± 0.46 | 5.89 ± 0.51 | 9.24 ± 1.27 |
| 10 % mepiquat chloride | 24.59 ± 0.77 | 6.25 ± 0.66 | 8.70 ± 1.52 |
| 25 % mepiquat chloride | 25.65 ± 0.57 | 6.46 ± 1.2 | 8.59 ± 2.82 |
| 50 % mepiquat chloride | 26.95 ± 1.31 | 6.09 ± 0.52 | 9.83 ± 1.16 |
| Vehicle (acetone/olive oil) | 25.43 ± 0.87 | 6.98 ± 1.05 | 10.07 ± 1.45 |
| Positive control | 29.35* ± 0.82 | 13.47* ± 1.33 | 27.53* ± 1.11 |

MEAN ± SD
*: Statistically significant vs. concurrent control

Report on the study of the sensitizing effect of mepiquat chloride in guinea pigs

This study was submitted to DAR (2005). It was pre-GLP regulations. There are several major deviations when comparing to OECD TG 406 (1992) and the test does not fully represent the Buehler or Magnusson and Kligman test either.

The preliminary test: 100 µl of the test substance was applied intracutaneously at concentrations of 10 %, 25 % and 50 %. Five Pirbright White guinea pigs were used per group. At concentrations of 10 % only very slight erythema were detected. At higher doses pronounced changes were evident: 2 wheals containing a 50 % preparation per animal caused the death of the test animals. 2 wheals containing a 25 % solution caused necrotic skin changes at the application site and in some cases also severe irritation. On the basis of the above findings a 10 % solution of the test substance was chosen for the induction and challenge phases.

Intracutaneous induction with a 10 % test substance caused erythema (mainly grade 1-2 but also grade 4 observed in conjunction with necrotic skin changes) and oedema; occasionally necrosis was found at the injection site in test animals. The intracutaneous challenge with a 10 % mepiquat chloride caused erythema and oedema both in the negative control group and in the test animals. In both groups spotted necrotic-like skin areas were observed. All the animals in negative control and test group, treated with 10 % mepiquat-chloride, had skin reactions.

The dose level selected for intracutaneous induction was too high as necrotic skin changes were observed. Same dose level was used for challenge phase, but considered too high. Dose that causes no irritation should be used.

### 10.7.2 Comparison with the CLP criteria

Substances may be allocated to one of the two sub-categories 1A or 1B by using a weight of evidence approach in accordance with the criteria given in CLP and on the basis of reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals. For Category 1, a stimulation index of three or more is considered a positive response in the local lymph node assay.

In a well conducted LLNA study (OECD TG 429, dRAR B.6.2.6, 2019) stimulation Indices (S.I.) of 1.1, 1.2, and 1.2 were determined with the test item so the proliferation index values did not increase 3 times over the control values. Compared with the aforementioned criteria, the LLNA study did not indicate a skin sensitising potential.

The report on the study of the sensitizing effect of Mepiquat chloride in guinea pigs according to “Proposed Rules” of EPA seemed not to be suitable to study skin sensitization of mepiquat chloride according to the CLP regulation (EC) No 1272/2008. The dose leveles selected for intracutaneous induction and challenge phase were too high as necrotic skin changes were observed. Dose that causes no irritation should be used. The text from the DAR has been amended and conclusions have changed. The study is not considered acceptable.

### 10.7.3 Conclusion on classification and labelling for skin sensitisation

Since no evidence of a skin sensitizing potential existed in the LLNA study, the data available indicates that mepiquat chloride does not require classification as skin sensitiser according to the CLP regulation (EC) No 1272/2008.

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin sensitisation based on a negative LLNA.

### Comments received during consultation

One MSCA agreed that the LLNA was negative but requested justification for the choice of the vehicle (ethanol/water) due to a concern about its wetting properties.

### Assessment and comparison with the classification criteria

Two skin sensitisation studies are available, an LLNA (2019) and a non-standard study in Guinea pigs (1979) that was considered unacceptable by the DS.

The LLNA was conducted according to OECD TG 429 and under GLP. Mepiquat chloride (solid, purity 98.1%) was dissolved in ethanol/water 3:7 (v/v) and applied at concentrations of 10%, 25% and 50%. The top concentration was selected based on a preliminary experiment where 25% and 50% solutions caused slight erythema (grade 1). Alpha-hexyl cinnamaldehyde in acetone/olive oil 4:1 (v/v) at 25% was a concurrent positive control.

RAC notes that according to the test guideline care should be taken to ensure that hydrophilic substances are incorporated into a vehicle system which wets the skin and does not immediately run off, by incorporation of appropriate solubilisers (e.g. 1% Pluronic L92). Wholly aqueous vehicles are to be avoided. As ethanol has good wetting properties and mepiquat chloride possesses surface activity, there is no concern about wetting properties of the test solutions.

The stimulation indices were 1.1, 1.2 and 1.2 at 10%, 25% and 50% respectively. Stimulation index in the positive control was 7.6. The study is negative.

The non-standard test in Guinea pigs (1979) employed 10 intracutaneous inductions over 3 weeks. Intracutaneous challenge was carried out 2 weeks after the last induction. A concentration of 10% was chosen for both induction and challenge based on results of a preliminary experiment. There was no positive control. Relatively severe skin reactions (up to necrosis) were observed in both the treated and the control group in the main experiment. As there was no increase in skin-fold thickness (edema) or erythema in the treated group compared to the control, the study was interpreted as negative by the study authors. RAC agrees with the DS to exclude this study from the assessment.

In summary, RAC agrees with the DS's proposal of **no classification for skin sensitization** based on a negative LLNA.

## 10.8 Germ cell mutagenicity

Not assessed in this dossier

## 10.9 Carcinogenicity

### Table 18: Summary table of animal studies on carcinogenicity.

↑↓ denote an increase or decrease in a parameter with respect to the control value; a.i.= active ingredient; m=male, f=female; statistical significance: * p <0.05, ** p <0.01

<table>
<tr><th>Method</th><th>Dose levels</th><th>Results</th><th>Reference</th></tr>
<tr><td>24-month chronic toxicity study (oral) in Wistar rats. OECD 452 (2009) According to GLP<br><br>Date performed<br><br>02/1991-02/1993<br><br>20 males, 20 females<br><br>Mepiquat chloride purity: 58%</td><td>0, 290, 2316 or 5790 ppm in relation to a.i. corresponding to<br><br>0, 13, 106 or 268 mg/kg bw/day in males<br><br>0, 18, 146 or 371 mg/kg bw/day in females</td><td><u>Non-neoplastic findings</u><br><br><u>5790 ppm (268 /371 mg/kg bw/day)</u><br><br>↓ Body weight gain, both sexes m: 15-19%**, (days 35-182), 17%* (termination), f: 12%** (day 126), 20%** (day 546)<br><br>Adrenal cortex: focus f: 16/20 (control 9/20), vacuolated cell foci f: 9/20 (control 3/20)<br><br>Ovaries: dilated bursa 4/49 (control 0/20)<br><br>Lungs: alveolar haemorrhage f: 8/20 (control 2/20)<br><br>Liver: ↓ absolute weight, both sexes, m: 11%, f: 11%<br><br>Kidneys ↓ absolute weight, m: 15%<br><br>Brain ↓ absolute weight, m: 4%*<br><br>Adrenal glands ↑ absolute weight, m: 59%<br><br><u>Neoplastic findings</u><br><br>
<table>
<tr><th rowspan="2">Organ/Tumour</th><th colspan="4">Males</th></tr>
<tr><th>Control</th><th>290 ppm</th><th>2316 ppm</th><th>5790 ppm</th></tr>
<tr><td>Brain<br>-meningioma<br>-schwannoma</td><td><br>0/20<br>0/20</td><td><br>0/8<br>0/8</td><td><br>0/2<br>0/2</td><td><br>1/20<br>1/20</td></tr>
<tr><td>Pancreas<br>-islet-cell adenoma<br>-islet-cell carcinoma</td><td><br>0/20<br>0/20</td><td><br>0/5<br>1/5</td><td><br>0/2<br>0/2</td><td><br>2/20<br>0/20</td></tr>
<tr><td>Spleen<br>-hemangiosarcoma</td><td><br>0/20</td><td><br>2/7</td><td><br>1/4</td><td><br>2/20</td></tr>
</table>
<table>
<tr><th></th><th>Females</th></tr>
</table>
</td><td>dRAR<br><br>B.6.5.1., 1994</td></tr>
</table>

<table>
<tr><th>Method</th><th>Dose levels</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><th>Organ/tumour</th><th>Control</th><th>290 ppm</th><th>2316 ppm</th><th>5790 ppm</th></tr>
<tr><td>Mammary glands<br>- fibroadenoma<br>- adenocarcinoma</td><td>3/20<br>0/20</td><td>2/8<br>2/8</td><td>3/11<br>2/2</td><td>4/20<br>2/20</td></tr>
</table>
</td><td></td></tr>
<tr><td>2-year carcinogenicity study OECD 451 (2009), minor deviations.<br>According to GLP<br>Date performed<br>02/1991-02/1993<br>Wistar rats<br>50 males, 50 females<br>Mepiquat chloride purity: 58%</td><td>0, 290, 2316 or 5790 ppm in relation to a.i. corresponding to<br>0, 13, 105 or 269 mg/kg bw/day in males<br>0, 17, 141 or 370 mg/kg bw/day in females</td><td><u>Non-neoplastic findings</u><br><u>5790 ppm (269 /370 mg/kg bw/day)</u><br>↓ Body weight gain, both sexes days 7-728, 18**-34**% f: days 7-728, 11**-33**%<br>Blood counts at termination<br>-Males:<br>↓EOS% 1.41 (62%), ↑ BASO% 0.85 (77%), ↓BAND% 0.10 (53%), ↓MONO% 10.41 (77%)<br>-Females:<br>↑ BASO% 0.85 (74%), ↑ BAND% 0.30 (100%), ↑ POLY% 27.00 (22%)<br>Morphological variations in blood cells at termination<br>-Changes in nucleus of lymphocytes<br>m:6/41 (control 1/27)<br>-Changes in plasma of lymphocytes<br>m: 7/41 (control 1/27)<br>-Changes in nucleus of monocytes<br>f: 5/37 (control 1/33)<br>Brain: ↑ Relative weight, m: 19%**, f: 20%**<br>Kidneys: ↓ Relative weight, f: 19%**<br>Adrenal gland: ↑ Relative weight f: 57%<br>Adrenal cortex, focus f: 39/50 (control 31/50)<br>Liver, cyst, f: 12/50 (control 7/50)<br>Ovaries, cyst f: 24/50 (control 14/50)<br>Ileum, lymphoid hyperplasia m: 5/46 (control 0/49)<br>Thyroid glands, C-cell hyperplasia m: 31/50 (control 18/50)<br>Ovaries, dilated bursa 13/50 (control 1/50)<br>Uterus/cervix, squamous hyperplasia 14/50 (control 5/50), stromal hyperplasia 11/50 (control 2/50)</td><td>dRAR<br>B.6.5.1., 1994</td></tr>
</table>

<table>
<tr><th>Method</th><th>Dose levels</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
Pituitary gland, cyst m: 7/50 (control 3/50), f: 10/50 (control 3/50), hyperplasia m: 13/50 (control 8/50), f: 6/50 (control 4/50)<br><br>
<u>2316 ppm (105/141 mg/kg bw/day)</u><br>
↓Body weight gain, m: 15%**, day 728<br>
Adrenal cortex, focus f: 39/50 (control 31/50)<br>
Liver, cyst, m: 12/50 (control 7/50)<br><br>
<u>Neoplastic findings</u>
<table>
<tr><th>Organ/Tumour</th><th colspan="4">Males</th></tr>
<tr><th></th><th>Control</th><th>290 ppm</th><th>2316 ppm</th><th>5790 ppm</th></tr>
<tr><td>Urinary bladder<br>-urothelial papilloma</td><td>1/50</td><td>0/15</td><td>2/17</td><td>3/50</td></tr>
<tr><td>Thyroid<br>-C-cell adenoma</td><td>4/50</td><td>0/12</td><td>3/15</td><td>6/50</td></tr>
<tr><td>Spleen<br>-hemangiosarcoma</td><td>1/50</td><td>5/20</td><td>3/17</td><td>1/50</td></tr>
<tr><td>Thymus<br>-thymoma</td><td>6/40</td><td>2/14</td><td>6/15</td><td>10/46</td></tr>
</table>
<table>
<tr><th>Organ/Tumour</th><th colspan="4">Females</th></tr>
<tr><th></th><th>Control</th><th>290 ppm</th><th>2316 ppm</th><th>5790 ppm</th></tr>
<tr><td>Brain<br>-glioblastoma</td><td>0/50</td><td>0/22</td><td>0/22</td><td>2/50</td></tr>
<tr><td>Uterus<br>-adenocarcinoma<br>-stromal polyp<br>-hemangiosarcoma</td><td>0/50<br>2/50<br>0/50</td><td>0/19<br>1/19<br>0/19</td><td>1/24<br>4/24<br>1/24</td><td>2/50<br>4/50<br>2/50</td></tr>
<tr><td>Liver<br>-carcinoma</td><td>0/50</td><td>3/50</td><td>1/50</td><td>2/50</td></tr>
</table>
</td><td></td></tr>
</table>

| Method | Dose levels | Results | Reference |
|---|---|---|---|
| | | | |

| Method | Dose levels | Results | Reference |
|---|---|---|---|
| 104-weeks oral toxicity study<br>Non-guideline<br>Non-GLP<br>Groups of 35, 50, 55 or 105 male and female Sprague Dawley rats<br>Mepiquat chloride purity: 94% | 0, 100, 300, 1000, 3000 and 9000 ppm corresponding to<br>0, 6, 18, 62, 186 and 684 mg/kg bw/day in males<br>0, 7, 21, 72, 212 and 670 mg/kg bw/day in females<br>0<br>0-2<br>0-5<br>0-16<br>0-4<br>0-5 0-5 | **Non-neoplastic findings**<br>9000 ppm (684 /670 mg/kg bw/day)<br>↓ Body weight gain, m: 11% f: 14%<br>*Heart* ↓ absolute weight, f: 14%*<br>*Liver* ↓ absolute weight, f: 10%*<br>*Kidneys* ↓ absolute weight, f: 10%*<br>*Adrenals* ↓absolute weight, m: 25%*<br>*Brain* ↓ absolute weight, m: 10%*<br>3000 ppm (186/212 mg/kg bw/day)<br>*Adrenals* ↓absolute weight, m: 17%*<br>1000 ppm (62/72 mg/kg bw/day)<br>*Adrenals* ↓absolute weight, m: 20%*<br><br>**Neoplastic findings**<br>None | dRAR<br>B.6.5.1., 1979 |
| 2-year carcinogenicity study<br>OECD 451 (2009)<br>According to GLP<br>B6C3F1 mice<br>10/40 males, 10/40 females<br>In satellite/<br>main group<br>Satellite group: 12 months exposure<br>Main group: 24 months exposure<br>Mepiquat chloride purity:58 % | 0, 500, 2000, 7500 ppm in relation to a.i.corresponding to<br>0, 74, 297 and 1140 mg/kg bw/day in males<br>0,85, 328 and 1348 mg/kg bw/day in females | **Non-neoplastic findings**<br>7500 ppm<br>*Monocytes* ↑, m: 49%<br>*Monocytes* ↓, f: 11%<br>*Lung* mass, m: 5/40 (control 2/40)<br>*Preputial glands*, enlarged, m: 13/40 (control 6/40)<br>*Pancreas*, hyperplasia, islet m: 10/40 (control 7/40), f: 5/40 (control 1/40)<br>*Forestomach*, focus *m: 4/40 (control 0/40)*<br>*Glandular stomach*, m: focus 3/30 (control 0/40)<br>*Kidneys*, vascuolization, m: 34/40, (control 22/40)<br>*Mesenteric lymph nodes*, hyperplasia, lymph m: 5/40 (control 3/40) f: 2/40 (control 1/40)<br>*Spleen*: hyperplasia, lymph f: 6/40 (control 3/40), hemosiderin storage 3/40 (control 1/40)<br>*Iliac lymph nodes*, hyperplasia: f: 7/40 (control 2/40)<br>*Pancreas*, hyperplasia, islet m: 10/40 (control 47/40, f: 5/40 (control 1/40)<br>2000 ppm<br>*Kidneys*, vacuolization, m:35/40 (control 22/40), tubular hyperplasia, m: 35/40 (control | dRAR<br>B.6.5.1., 1994 |

| Method | Dose levels | Results | Reference |
|---|---|---|---|
| | | 25/40), f: 4/40 (control 1/40)<br><u>500 ppm</u><br>*Forestomach*, focus m: 5/40 (control 0/40)<br><br>**<u>Neoplastic findings</u>**<br>**Tumour incidences at 0, 500, 2000 and 7500 ppm**<br>Males (n=50)<br><u>Lung adenocarcinomas</u><br>4%, 4%, 2%, 8% (*HCD: 0-14%)<br><u>Lung adenomas</u><br>12%, 6%, 14%, 14% (*HCD: 0-12%)<br>Females (n=50)<br><u>Lung adenocarcinomas</u><br>2%, 2%, 0%, 4%, (*HCD: 0-2%)<br><u>Lung adenomas</u><br>2%, 4%, 2%, 2% (*HCD: 0-6%)<br><u>Liver, hepatocellular adenomas</u><br>8%, 8%, 8%. 2% (*HCD: 6-16%)<br><u>Liver, hepatocellular carcinomas</u><br>2%, 0%, 6%, 6% (*HCD: 2-6%)<br>')HCD from two studies, 100 animals | |
| 2-year chronic toxicity and carcinogenicity study in NMRI mice<br>Non-guideline<br>Non-GLP<br>Control animals: n=100<br>Test groups:<br>50 animals /sex<br><br><br>Mepiquat chloride purity: 94% | 0, 100, 300, 1000, 3000 ppm corresponding to<br>0, 16, 48.9, 169.4 and 513.5 mg/kg bw/day in males<br>0, 21.7, 65.3, 226.1 and 689.4 mg/kg bw/day in females | **<u>Non-neoplastic findings</u>**<br><u>3000 ppm</u><br>*Leucocytes* ↑ m: 19-32% at weeks 26-104<br>*Spleen*, ↑ relative weight, m: 27%, f: 19%<br>*Spleen*, ↑absolute weight, m: 26%<br>*Thymus*, ↓relative weight, f: 73%<br><u>1000 ppm</u><br>*Leucocytes* ↑ m: 119-126% at weeks 26-104<br>*Spleen*, ↓relative weight, m: 13%,<br>*Spleen*, ↓ absolute weight, m: 16%<br><u>300 ppm</u><br>*Leucocytes* ↑ m: 112-120% at weeks 26-104<br>*Spleen*, ↓relative weight, m: 21%,<br>*Spleen*, ↓ absolute weight, m: 21%<br><u>100 ppm</u><br>*Leucocytes* ↑ m: 130%** at week 52 | dRAR B.6.5.1., 1979 |

| Method | Dose levels | Results | Reference |
|---|---|---|---|
| | | *Spleen*, ↓ relative weight, m: 13%,<br>*Spleen*, ↓ absolute weight, m: 11%<br><br>**<u>Neoplastic findings</u>**<br>**Tumour incidences at 0, 100, 300, 1000 and 3000 ppm**<br>Males<br>Lymphomas: 1%, 2%, 4%, 2%, 4%<br>Adenoma, pituitary: 0%, 0%, 2%, 0%, 0%<br><br>Females<br>Lymphomas: 1%, 2%, 3%, 0%, 8%<br>Adenoma, pituitary: 1%, 0%, 0%, 2%, 4%<br>Leiomyoma, uterus: 0%, 0%, 2%, 0%, 4%<br>Necrotic tumour ovary: 0%, 0%, 0%, 0%, 4%<br><br>-HCD not available for the study | |

**Table 19: Summary table of human data on carcinogenicity**

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

**Table 20: Summary table of other studies relevant for carcinogenicity. Summary of findings of the genotoxicity studies.**

| Method, guideline, deviations if any | | Test substance | Organism/strain | Concentrations tested | Results/obervations | Reference |
|---|---|---|---|---|---|---|
| | ***In vitro* studies** | | | | | |
| <u>Bacterial reverse gene mutation assay (Ames test)</u><br>OECD 471<br>GLP<br>Highest dose below the recommended dose.<br>Shortcomings in selection of control substances | | Mepiquat chloride, purity 99.8 | *Salmonella typhimurium* strains TA 98, TA100, TA1535, TA1537, TA1538 | 4, 20, 100, 500 and 2500 µg/plate with and without S9 | Negative | dRAR B.6.4.1., 1979 |

| **Method, guideline, deviations if any** | | **Test substance** | **Organism/strain** | **Concentrations tested** | **Results/obervations** | **Reference** |
|---|---|---|---|---|---|---|
| Bacterial reverse gene mutation assay (Ames test)<br><br>OECD 471 (1997)<br><br>GLP | | Mepiquat chloride, purity 99.6% | *Salmonella typhimurium* strains TA 98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 uvrA | 156.3, 312.5, 625, 1250, 2500 and 5000 µg/plate, with and without S9 mix | Negative | dRAR B.6.4.1., 1990 |
| DNA repair test on bacteria<br><br>According to JMAFF guidelines<br><br>GLP | | Mepiquat chloride, purity 99.6% | *Bacillus subtilis* strains H17 and M45 | Without S9: from 2484 µg/disk to 39740 µg/disk<br><br>With S9: from 1242 µg/disk to 19870 µg/disk | Negative | dRAR B.6.4.1., 1990 |
| *In vitro* gene mutation test<br><br>(HPRT locus assay)<br><br>OECD 476 with deviations.<br><br>325.0, 650, 1300 and 2600 µg/ml with and without S9 mix<br><br>In 1st experiment S9 fraction:cofactors was 3:7, 2nd experiment 1:9<br><br>GLP | | Mepiquat chloride, purity 617.6 g/l | CHO-cells | 162.5, 325, 650, 1300 and 2600 µg/ml (10mM) | Negative<br><br>In dRAR the study is considered not acceptable. | dRAR B.6.4.1., 2002 |
| *In vitro* chromosomal aberration study<br><br>OECD 473 (1997, 2016) with deviations in exposure duration and sampling times | | Mepiquat chloride, purity >99% | Chinese hamster ovary (CHO-WBI) cells | 2.0, 3.0, 4.0 and 5.0 mg/ml,<br><br>with and without S9 mix with for 8 h with S9 mix and 2 h without S9 mix. (in guideline 3-6 h for both experiments)<br><br>Sampling in the | Negative<br><br>In dRAR the study is considered not acceptable. | dRAR B.6.4.1., 1987 |

| **Method, guideline, deviations if any** | | **Test substance** | **Organism/strain** | **Concentrations tested** | **Results/obervations** | **Reference** |
|---|---|---|---|---|---|---|
| | | | | main test was done app. at 10 h | | |
| Unscheduled DNA synthesis (UDS) assay<br>OECD 482<br>GLP | | Mepiquat chloride, purity >99% | Rat hepatocytes | 25.6, 51.2, 102, 256, 512, 1020, 1000, 2,000 and 3000 µg/ml | Negative | dRAR B 6.4.1., 1987 |
| This study is new. The study was performed in line with OECD Guidelines for Testing of Chemicals No. 487 "*In Vitro* Mammalian Cell Micronucleus Test", adopted 29 July 2016 in accordance with GLP (certified laboratory). | | 60.9% (in aqueous solution); N-methylpiperidine: 0.179% | Normal human lymphocytes | 0, 46.88, 93.75, 187.5, 375, 750, 1500 µg/mL. | Mepiquat chloride technical concentrate was considered to be non-clastogenic and non-aneugenic to human lymphocytes *in vitro*. | dRAR B 6.4.1., 2019 |
| | ***In vivo* studies in somatic cells** | | | | | |
| Mouse bone marrow micronucleus test<br>OECD 474 (1997, 2016)<br>GLP | | Mepiquat chloride, purity 617.6 g/L | NMRI mice<br>5 males/group | 0, 250, 500, 750 and 1000 mg/kg bw<br>Two oral doses at 24 h interval | Negative<br>Two animals of the highest dose group died after the 1st administration<br>The study was amended by an additional ADME experiment (OECD TG417) on exposure of bone marrow in mouse, which showed evidence of bone marrow exposure one hour after last administration. | dRAR B 6.4.2., 2002<br>dRAR B 6.4.2., 2016 |
| Dominant lethal assay<br>Non-GLP | | Mepiquat chloride, purity 94% | NMRI mice<br>20 males/group<br>60 females/group | 0, 26.1, 78.5, 268.2 and 816 mg/kg bw | Negative | dRAR B 6.4.2., 1977 |

**Rat**

Chronic toxicity study (OECD 452)

Groups of 20 male and 20 female Wistar rats were administered mepiquat chloride in the diet for up to 24 months at concentrations of 0, 290, 2316, or 5790 ppm in relation to a.i. (corresponding to achieved daily intakes of 0, 13, 106 or 268 mg/kg bw/day in males and 0, 18, 146, or 371 mg/kg bw/day in females)

The study was conducted in according to OECD guideline 452 (2009) with following deviations: clinical observations/neurofunctional observations were made less often than according to OECD guideline. At necropsy the weights of epididymides, heart, ovaries, spleen, thyroid and uterus were not measured.

Food consumption and body weight were determined weekly for 14 weeks and every four weeks thereafter. The general state of animal health was checked daily. Detailed clinical examinations of the animals were performed once a week. The first 10 animals of each group were subjected to neurofunctional observations before the start of treatment, and at about 1, 2, 3 and 6 months after the start of the administration period. The functional observation battery consisted of following parameters: general condition, animal body, piloerection, skin colour, posture, respiration, behavior, activity, tremors, convulsions, ataxia, paresis/paralysis, pupil size, lacrimation, secration of pigmented tears, salivation, vocalization, body tone, urination, feces, sensitivity of the body surface, righting response, winking reflex, pupillary reflex, vision, audition, olfaction, toe pinch, tail pinch and miscellaneous. Also quantitative parameters (grip strength of fore- and hindlimbs and hot-plate test) were examined.Haematology, clinical chemical examinations and urinalysis were conducted after about 3, 6, 12, 18 and 24 months on all surviving animals per test group and sex. Hematological parameters investigated were: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, differential blood count, reticulocytes and prothrombin. Also, differential blood count was performed. Clinical chemical examinations included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum-γ-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol and magnesium. Urinalyses included volume, color, turbidity, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity and sediment. Ophthalmological examinations were carried out once before the start of the study and towards the end of the administration period on control and high dose groups. After 24 months of treatment all surviving rats were sacrificed. All animals were subjected to complete grosspathological examinations. The weights of adrenal glands, brain, kidneys, liver and testes were determined. Histopathological examinations were performed on all animals of control and high dose groups. In addition lungs, liver and kidneys were examined for all animals on low and mid dose groups. Gross lesions were examined for all animals affected. Histopathological examination was performed similarly for animals of low and mid dose groups which died or were killed in extremis as done for the high dose group at study termination.

**Results**

**Non-neoplastic findings**

In neurological examinations no effects were observed at any time point of the study. No statistically significant differences in the grip strength of forelimbs on males and females and the changes observed were within 10% of control value. No consistent treatment-related changes were found in haematology and clinical chemistry analyses. The ophthalmological examinations revealed no significant findings. No significant treatment-related effect on mortality was observed.

**Table 21 Mortality rates (%).**

| **Males** | | | | **Females** | | | |
|---|---|---|---|---|---|---|---|
| **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** |
| 25% | 30% | 5% | 30% | 25% | 25% | 30% | 25% |

Food consumption was slightly impaired at 5790 ppm in both sexes. In males of the high dose group, body weight and body weight change were statistically significantly reduced from study day 7 onwards. There was a reduction in terminal body weight of about 12 % and in terminal body weight gain of about 17 % in the high dose group compared with control values. In females of the high dose group, body weight was statistically significantly decreased from study day 98 onwards until day 574 and terminal body weight was about 12 % below control values. Terminal body weight gains were 20 % below control levels.

**Table 22 Body weight values (g).**

| Day | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** |
| **35** | 186.1 | 184.5 | 181.2 | 150.5** (81%) | 80.2 | 84.0 | 79.3 | 72.9 |
| **126** | 341.3 | 345.1 | 331.0 | 284.0** (83%) | 149.9 | 155.9 | 145.0 | 131.4** (88%) |
| **546** | 525.1 | 520.9 | 500.4 | 446.4* (85%) | 219.8 | 224.0 | 207.8 | 176.0** (80%) |
| **728** | 521.2 | 510.8 | 466.0 (89%) | 434.3* (83%) | 235.6 | 216.9 | 230.6 | 188.6 (80%) |

(Values in parentheses are the % of the control value.)

*p<0.05, **p<0.01

Macroscopical examinations showed increased number of adrenal cortex focus in males and females. Microscopic examinations at termination showed increased number of alveolar hemorrhage in lung in high dose females. In adrenal cortex increased number of vacuolated cell foci in high dose group females and cellular hypertrophy in high dose males was observed. In all treated females dilated bursa in ovaries was observed.

**Table 23 Macroscopical and microscopical findings.**

| Organ | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** |
| **Macroscopical findings** | | | | | | | | |
| **Adrenal cortex**<br>-focus | 2 | 3 | 6 | 4 | 9 | 13 | 3 | 16 |
| **Microscopical findings** | | | | | | | | |
| **Lungs**<br>**no. examined**<br>-alveolar hemorrhage | 20<br>6 | 20<br>8 | 20<br>10 | 20<br>6 | 20<br>2 | 20<br>3 | 20<br>6 | 20<br>8 |
| **Adrenal coxtex**<br>**no.examined**<br>-vacuolated cell foci<br>-cellular hypertrophy | 20<br>4<br>7 | 9<br>1<br>2 | 7<br>3<br>3 | 20<br>1<br>12 | 20<br>3<br>7 | 17<br>5<br>6 | 18<br>5<br>5 | 20<br>9<br>2 |
| **Ovaries**<br>**no. examined**<br>-dilated bursa | - | - | - | - | 20<br>- | 8<br>2 | 12<br>1 | 20<br>4 |

**Neoplastic findings**

An overall summary of incidencies of neoplasms in control and treated groups is provided in table. There was no significant difference in incidencies of benign and malignant tumours between control and treated groups.

**Table 24 Total number of benign and malignant neoplasms.**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **Dose (ppm)** | **0** | **290** | **2316** | **5790** | **0** | **290** | **2316** | **5790** |
| **No of animals** | **20** | **20*** | **20*** | **20** | **20** | **20*** | **20*** | **20** |
| **Total number** | | | | | | | | |
| Benign neoplasms | 37 | 29 | 24 | 29 | 36 | 27 | 32 | 24 |
| Malignant neoplasms | 8 | 7 | 8 | 12 | 3 | 6 | 9 | 7 |

*) Not all organs were examined.

**Table 25 Incidencies of primary neoplasms.**

| **Organ** | **Males** | | | | **Females** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** |
| **Brain** | | | | | | | | |
| **no. examined** | 20 | 8 | 2 | 20 | 20 | 10 | 10 | 20 |
| -meningioma | - | - | - | 1 | - | - | - | - |
| -schwannoma | - | - | - | 1 | - | - | - | - |
| **Liver** | | | | | | | | |
| **no. examined** | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| -cholangioma | - | - | - | 1 | - | - | 1 | - |
| -carcinoma | 1 | 1 | 1 | 2 | - | - | 1 | 1 |
| **Mammary glands** | | | | | | | | |
| **no. examined** | - | - | - | - | 20 | 8 | 11 | 20 |
| -fibroadenoma | | | | | 3 | 2 | 3 | 4 |
| -adenocarcinoma | | | | | - | 2 | 2 | 2 |
| **Thyroid glands** | | | | | | | | |
| **no. examined** | 20 | 6 | 2 | 20 | 20 | 5 | 8 | 20 |
| -C-cell adenoma | - | - | - | 1 | 2 | 1 | 1 | 2 |
| **Pancreas** | | | | | | | | |
| **no. examined** | 20 | 5 | 2 | 20 | 20 | 7 | 7 | 19 |
| -islet-cell adenoma | - | - | - | 2 | - | - | - | - |
| -islet-cell carcinoma | - | 1 | - | - | - | - | - | - |
| **Uterus** | - | - | - | - | | | | |
| **no. examined** | | | | | 20 | 14 | 9 | 20 |
| -leiomyoma | | | | | - | - | - | 1 |
| **Spleen** | | | | | | | | |
| **no. examined** | 20 | 7 | 4 | 20 | 20 | 5 | 7 | 20 |
| -hemangiosarcoma | - | 2 | 1 | 2 | - | - | 1 | - |
| **Thymus** | | | | | | | | |
| **no. examined** | 19 | 7 | 2 | 17 | 17 | 5 | 10 | 19 |
| -thymoma | 7 | 1 | - | 1 | 4 | 2 | 4 | 2 |

For individual tumour types there were slightly increased frequencies in test animals as compared of those seen in concurrent controls. Types of tumours showing 1-2 cases over the control among the high dose groups were: brain meningioma and schwannoma, thyroid gland C-cell adenoma, pancreas cell adenomas and carcinoma and chlolangioma of liver and spleen hemangiosarcoma in males and mammary gland fibroadenomas and adenocarcinomas and uterus leiomyomas in females.

Same rat strain from same supplier with a larger number of animals was used in the carcinogenicity study. Moreover, the two studies were performed parallel. Therefore, it was considered justified to combine data on neoplasias showing elevations in incidencies in treated rats to better distinguish between spontaneuous neoplasias and possible treatment-related ones. The available HCD, applicable to both studies, is used in evaluation of relevance of tumour findings. The combined data on observed tumours of rat chronic toxicity and carcinogenicity studies are presented in table 33

Carcinogenicity study (OECD 451)

The study was conducted in accordance with GLP provisions and was mainly conducted in accordance with OECD guideline 451 (2009). The dose interval between low and mid doses is greater than 2-4 fold interval mentioned in the guideline.

Groups of 50 male and 50 female Wistar rats were administered mepiquat chloride in the diet for up to 24 months at concentrations of 0, 290, 2316, and 5790 ppm corresponding to achieved daily intakes of 0, 13, 105 and 269 mg/kg bw/day in males and 0, 17, 141, and 370 mg/kg bw/day in females.

Feed consumption and body weight were determined weekly for 14 weeks and every four weeks thereafter. The general state of animal health and mortality were checked daily. Detailed clinical examinations of the animals were performed once a week. Blood was collected for differential blood smears at the termination of the study from control and high dose group animals and additionally from all animals killed in extremis during the study.

Complete necropsy was performed on all rats for all surviving rats at the end of the study. Organ weights of the brain, liver, kidneys, adrenal glands and testes were recorded. Gross lesions were examined for all animals affected. At the end of study histopathological examinations for 43 organs were performed on all animals for control and high dose groups as well as on those low and mid dosed animals which died or were killed in extremis during the study. Lungs, liver and kidneys were examined for all animals. In addition, lungs, liver and kidneys were examined for all animals of low and mid dose groups. Statistical assessment of organ weights and terminal body weight parameters was by the Dunnett test for the simultaneous comparison of dose groups.

At the end of study mortality was highest among control groups of both males and females.

**Table 26 Mortality.**

| Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|
| **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** |
| 23 (46%) | 12 (24%) | 14 (28%) | 9 (18%) | 17 (34%) | 11 (22%) | 10 (20%) | 13 (26%) |

Food consumption was reduced by 15% in males and 11% in females of high dose groups.

Statistically significant reduction in body weight was observed in high dose group males during the whole study period. In males of mid dose group body weight was occasionally statistically significantly reduced during days 0-266 and from study day 294 onwards until the end of the study. In low dose groups of males body weight was occasionally statistically significantly reduced during the study. Among females, statistically significant reduction in body weight was observed in high dose group from study day 7 onwards until the end of the study.

**Table 27 Body weights on days 35-728.**

| Day | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 290 ppm | 2316 ppm | 5790 ppm | Control | 290 ppm | 2316 ppm | 5790 ppm |
| **35** | 197.9 | 194.3 | 187.7* | 159.1** (80%) | 82.3 | 87.6 | 85.9 | 72.9** (89%) |
| **126** | 377.3 | 367.6 | 358.1* | 306.5** (81%) | 161.0 | 158.1 | 155.9 | 132.4** (82%) |
| **546** | 586.8 | 547.0* | 538.1** | 454.8** (78%) | 248.6 | 234.8 | 228.6 | 186.2** (75%) |
| **728** | 597.3 | 531.8** (89%) | 508.4** (85%) | 459.8** (77%) | 259.2 | 269.3 | 253.0 | 184.0** (71%) |

(Values in parentheses are the % of the control value.)

*p<0.05, **p<0.01

Further information regarding body weights was presented in the DAR (2015):

"The Notifier submitted that the reduced body weights in males of the mid and low dose groups were not treatment-related for the following reasons: comparison of the growth curves of these males with the ones of the chronic toxicity study (which was performed in parallel with the carcinogenicity study) no obvious biologically relevant differences could be noted. The body weight curve of the control animals of the carcinogenicity study showed a different pattern from what was considered normal; an increase in body weight was observed in the second year of the study. This increase in mean body weight in aged rats is unusual and in fact not related to a general increase in all animals but rather to the intercurrent death of several control animals with low body weights resulting in an increase of mean control body weight. Statistical significance was thus obtained due to an increase in mean body weight of controls and (due to the elimination of animals with low body weights) a reduction of standard deviation in this group.

Differential blood counts revealed changes between high dose and control groups both in males and females. The changes remained statistically insignificant.

**Table 28: Differential blood counts at termination.**

| Parameter | Males | | Females | |
|---|---|---|---|---|
| | Control | 5790 ppm | Control | 5790 ppm |
| **EOS %** | 2.26 | 1.41 (62%) | 1.70 | 1.57 |
| **BASO %** | 0.48 | 0.85 (177%) | 0.42 | 0.73 (174%) |
| **BAND %** | 0.19 | 0.10 (53%) | 0.15 | 0.30 (200%) |
| **MONO %** | 13.48 | 10.41 (77%) | 11.85 | 12.03 |
| **POLY %** | 17.52 | 16.39 | 22.06 | 27.00 (122%) |

Values in parentheses are % of control- values.

Morphological variations were detected at the end of the study (table) at the highest dose levels.

**Table 29: Morphological variations in white blood cells at termination of the study.**

| Target of variation | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 290 ppm | 2316 ppm | 5790 ppm | Control | 290 ppm | 2316 ppm | 5790 ppm |
| Nucleus of lymphocytes | 1/27 | - | - | 6/41 | 2/33 | - | - | 2/37 |
| Plasma of lymphocytes | 1/27 | - | - | 7/41 | - | - | - | - |
| Nucleus of monocytes | - | - | - | - | 1/33 | - | - | 5/37 |

The relative weights of brains of males and females and kidneys of females on high dose group were increased compared to control. In females of the high dose group relative weight of adrenals was elevated.

**Table 30: Organ weights of brain, kidneys and adrenal glands.**

| Organ | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 290 ppm | 2316 ppm | 5790 ppm | Control | 290 ppm | 2316 ppm | 5790 ppm |
| **Relative weight (%)** | | | | | | | | |
| Brain | 0.306 | 0.355 (116%) | 0.353 (115%) | 0.365* (119%) | 0.562 | 0.543 | 0.56 | 0.672** (120%) |
| Kidneys | 0.619 | 0.648 | 0.664 | 0.64 | 0.746 | 0.737 | 0.759 | 0.89** (119%) |
| Adrenal glands | 0.029 | 0.014 (48%) | 0.015 (52%) | 0.015 (52%) | 0.054 | 0.037 (69%) | 0.057 | 0.085 (157%) |

In macroscopical examinations an elevated incidence of focus in adrenal cortex in males of the high dose group was observed. Increased incidence of cyst in liver and ovaries was observed on females at all dose groups compared to controls. In histological examination increased incidence of lymphoid hyperplasia in ileum was seen in males of the high dose group. Females at top dose showed an increased incidence of dilated bursa in ovaries and uterus/cervix squamous hyperplasia and stromal hyperplasia. In both males and females of high dose group increased incidence of pituitary gland cysts and in males also pituitary gland hyperplasia were observed. In males of high dose group increased incidence of alveolar distention of seminal vesicles was found. In all treated males a higher incidence of alveolar distention of seminal vesicles and alveolar atrophy of prostate was observed.

**Table 31: Incidence of macroscopical and microscopical findings.**

| Organ/tissue | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 290 ppm | 2316 ppm | 5790 ppm | Control | 290 ppm | 2316 ppm | 5790 ppm |
| **Macroscopical findings** | | | | | | | | |
| **Adrenal cortex** | | | | | | | | |
| -focus | 5 | 10 | 7 | 16 | 31 | 36 | 39 | 39 |
| **Ovaries** | | | | | | | | |
| -cyst | - | - | - | - | 14 | 20 | 16 | 24 |
| **Liver** | | | | | | | | |
| -cyst | 1 | 3 | 1 | 1 | 7 | 12 | 15 | 12 |
| -focus | 27 | 37 | 31 | 31 | 20 | 23 | 11 | 18 |
| **Microscopical findings** | | | | | | | | |
| **Ileum** | | | | | | | | |
| **no. examined** | 49 | 10 | 10 | 46 | 46 | 10 | 10 | 47 |
| -lymphoid hyperplasia | - | - | - | 5 | 1 | - | - | - |
| **Ovaries** | | | | | | | | |
| **no. examined** | - | - | - | - | 50 | 31 | 27 | 50 |
| -dilated bursa | | | | | 1 | 2 | 2 | 13 |
| -stromal fibrosis | | | | | 1 | 1 | 1 | 5 |
| **Uterus/cervix** | | | | | | | | |
| **no. examined** | - | - | - | - | 50 | 19 | 24 | 50 |
| -squamous hyperplasia | | | | | 5 | 5 | 4 | 14 |
| -stromal hyperplasia | | | | | 2 | 3 | 1 | 11 |
| **Thyroid glands** | | | | | | | | |
| **no. examined** | 50 | 12 | 15 | 50 | 50 | 11 | 11 | 50 |
| -C-cell hyperplasia | 18 | 4 | 3 | 31 | 24 | 7 | 3 | 17 |
| **Pituitary gland** | | | | | | | | |
| **no. examined** | 50 | 21 | 20 | 50 | 50 | 42 | 41 | 50 |
| -cyst(s) | 3 | - | 3 | 7 | 3 | 2 | 6 | 10 |
| -hyperplasia | 8 | 3 | 4 | 13 | 4 | 5 | 3 | 6 |
| **Seminal vesicles** | | | | | | | | |
| **no. examined** | 50 | 20 | 19 | 50 | - | - | - | - |
| -alveolar distention | 0 | 2 | 2 | 4 | | | | |
| **Prostate** | | | | | | | | |
| **no. examined** | 50 | 20 | 18 | 50 | - | - | - | - |
| -alveolar atrophy | 1 | 2 | 4 | 7 | | | | |

**Neoplastic findings**

There were no statistically increased incidences in tumours in treated animals as compared with control animals. Slight, typically 1-2 cases above concurrent control, were observed both among treated males and females.

In males, increased incidence of urothelial papilloma, thyroid gland C-cell adenoma, thymoma, adrenal cortex adenoma, mammary gland fibroadenoma and adenocarcinoma, testes hemangioma and spleen hemangiosarcoma were found.

In females, elevated incidence of brain glioblastoma and granular cell tumour, uterus adenocarcinoma, uterine stromal polyp and hemangiosarcoma, adrenal cortex adenoma, mammary gland adenoma, adenocarcinoma, mammary cystadenoma and fibromas, spleen hemangioma, liver carcinoma and pancreas islet-cell carcinoma.

**Table 32: Incidence of primary neoplasms in rat carcinogenicity study.**

| Organ | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 290 ppm | 2316 ppm | 5790 ppm | Control | 290 ppm | 2316 ppm | 5790 ppm |
| **Brain** | | | | | | | | |
| **no. examined** | 50 | 13 | 15 | 50 | 50 | 22 | 22 | 50 |
| -glioblastoma | - | - | - | - | - | - | - | 2 |
| -granular cell tumour | 1 | 1 | - | 1 | - | - | - | 1 |
| -glioma | - | - | - | - | 1 | - | - | - |
| -oligodendroglioma | - | - | - | - | 1 | - | - | - |
| **Urinary bladder** | | | | | | | | |
| **no. examined** | 50 | 15 | 17 | 50 | 50 | 11 | 9 | 50 |
| -urothelial papilloma | 1 | - | 2 | 3 | - | - | - | - |
| **Uterus/cervix** | | | | | | | | |
| **no. examined** | - | - | - | - | 50 | 19 | 24 | 50 |
| -adenocarcinoma | | | | | - | - | 1 | 2 |
| -stromal polyp | | | | | 2 | 1 | 4 | 4 |
| -hemangiosarcoma | | | | | - | - | 1 | 2 |
| **Thyroid glands** | | | | | | | | |
| **no. examined** | 50 | 12 | 15 | 50 | 50 | 11 | 11 | 50 |
| -C-cell adenoma | 4 | - | 3 | 6 | 7 | 4 | 1 | 6 |
| **Thymus** | | | | | | | | |
| **no. examined** | 40 | 14 | 15 | 46 | 46 | 16 | 12 | 45 |
| -thymoma | 6 | 2 | 6 | 10 | 10 | 5 | 4 | 6 |
| **Adrenal cortex** | | | | | | | | |
| **no. examined** | 49 | 23 | 23 | 50 | 50 | 42 | 47 | 50 |
| -adenoma | 1 | 1 | - | 2 | - | 1 | 2 | 1 |
| **Mammary glands** | | | | | | | | |
| **no. examined** | - | - | - | 2 | 47 | 16 | 14 | 50 |
| -fibroadenoma | | | | 1 | 6 | 3 | 3 | 5 |
| -adenocarcinoma | | | | 1 | 4 | 1 | 3 | 6 |
| -adenoma | | | | - | - | - | 1 | - |
| -cystadenoma | | | | - | - | 2 | 1 | 1 |
| -fibroma | | | | - | - | - | 2 | - |
| -hemangiosarcoma | | | | - | 1 | - | - | - |
| **Testes** | | | | | | | | |
| **no. examined** | 50 | 39 | 39 | 50 | - | - | - | - |
| -hemangioma | - | - | 1 | 1 | | | | |
| **Spleen** | | | | | | | | |
| **no. examined** | 50 | 20 | 17 | 50 | 50 | 14 | 11 | 50 |
| -hemangioma | 2 | 1 | - | - | - | 1 | - | - |
| -hemangiosarcoma | 1 | 5 | 3 | 1 | 3 | - | - | 1 |
| **Liver** | | | | | | | | |
| **no. examined** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| -carcinoma | 5 | 2 | 3 | 3 | - | 3 | 1 | 2 |
| -hemangiosarcoma | 1 | - | - | - | 1 | 1 | 1 | 1 |
| **Pancreas** | | | | | | | | |
| **no. examined** | 50 | 15 | 14 | 50 | 50 | 14 | 12 | 50 |
| -islet-cell adenoma | 2 | - | - | 1 | - | - | - | - |
| -islet-cell carcinoma | - | 1 | - | - | - | 2 | - | - |
| -acinar adenoma | 3 | 2 | - | 1 | - | - | - | - |

In the chronic toxicity study of Wistar rat small elevations in the incidences of neoplasms in treated animals were found. As both studies were performed parallel with same rat strain in the same laboratory under similar conditions it was found justified to combine the tumour data from both studies (Table 33). The tumour incidencies with the HCD are indicated in Tables 34 and 35.

Table 33: Incidence of primary neoplasms in rat chronic and carcinogenicity studies.

| **Organ** | **Males** | | | | **Females** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** | **Control** | **290 ppm** | **2316 ppm** | **5790 ppm** |
| **Brain** | | | | | | | | |
| **no. examined** | 70 | 21 | 17 | 70 | 70 | 32 | 32 | 70 |
| -meningioma | - | - | - | 1 | - | - | - | - |
| -schwannoma | - | - | - | 1 | - | - | - | - |
| -glioblastoma | - | - | - | - | - | - | - | 2 |
| -granular cell tumour | 1 | 1 | - | - | - | - | - | 1 |
| -glioma | - | - | - | - | 1 | - | - | - |
| -oligodendroglioma | - | - | - | - | 1 | - | - | - |
| **Liver** | | | | | | | | |
| **no. examined** | 70 | 70 | 70 | 70 | 70 | 70 | 70 | 70 |
| -cholangioma | - | - | - | 1 | - | - | 1 | - |
| -carcinoma | 6 | 3 | 4 | 3 | - | 1 | 2 | 3 |
| -hemangiosarcoma | 1 | - | - | - | 1 | 1 | 1 | 1 |
| **Testes** | | | | | | | | |
| **no. examined** | 70 | 54 | 53 | 70 | - | - | - | - |
| -interst. cell tumour | 9 | 9 | 9 | 8 | | | | |
| -hemangioma | - | - | 1 | 1 | | | | |
| **Adrenal cortex** | | | | | | | | |
| **no. examined** | 69 | 32 | 30 | 70 | 70 | 59 | 65 | 70 |
| -adenoma | 1 | 2 | 1 | 2 | 1 | 3 | 3 | 1 |
| **Mammary glands** | | | | | | | | |
| **no. examined** | - | - | - | 2 | 67 | 24 | 25 | 70 |
| -fibroadenoma | | | | 1 | 9 | 5 | 6 | 9 |
| -adenocarcinoma | | | | 1 | 4 | 3 | 5 | 8 |
| -adenoma | | | | | - | - | 1 | - |
| -cystadenoma | | | | | - | 2 | 1 | 1 |
| -fibroma | | | | | - | - | 2 | - |
| -hemangiosarcoma | | | | | 1 | - | - | - |
| **Thyroid glands** | | | | | | | | |
| **no. examined** | 70 | 18 | 17 | 70 | 70 | 16 | 19 | 70 |
| -C-cell adenoma | 4 | - | 3 | 7 | 9 | 5 | 2 | 8 |
| **Pancreas** | | | | | | | | |
| **no. examined** | 70 | 20 | 16 | 70 | 70 | 21 | 19 | 69 |
| -islet-cell adenoma | 2 | - | - | 3 | - | - | - | - |
| -islet-cell carcinoma | - | 2 | - | - | - | 2 | - | - |
| -acinar adenoma | 3 | 2 | - | 1 | - | - | - | - |
| **Uterus** | - | - | - | - | | | | |
| **no. examined** | | | | | 70 | 33 | 33 | 70 |
| -adenocarcinoma | | | | | - | 1 | 1 | 2 |
| -stromal polyp | | | | | 2 | 1 | 4 | 4 |
| -leiomyoma | | | | | - | - | - | 1 |
| -hemangiosarcoma | | | | | - | - | 1 | 2 |
| **Thymus** | | | | | | | | |
| **no. examined** | 59 | 21 | 17 | 63 | 63 | 21 | 22 | 64 |
| -thymoma | 13 | 3 | 6 | 11 | 14 | 7 | 8 | 8 |
| **Spleen** | | | | | | | | |
| **no. examined** | 70 | 27 | 21 | 70 | 70 | 19 | 18 | 70 |
| -hemangiosarcoma | 1 | 7 | 4 | 3 | 3 | - | 1 | 1 |
| -hemangioma | 2 | 1 | - | - | - | 1 | - | 1 |
| **Ovaries** | | | | | | | | |
| **no. examined** | - | - | - | - | 70 | 39 | 59 | 70 |
| -sertoli cell tumour | | | | | - | - | - | 1 |
| -gonadal stroma tumour | | | | | - | - | - | 1 |
| **Urinary bladder** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **no. examined** | 70 | 21 | 19 | 70 | 70 | 16 | 16 | 70 |
| -urothelial papilloma | 1 | - | 2 | 3 | - | - | - | - |

The rat carcinogenicity study was performed during period of 02.91-02.93. HCD data available comprise a time period 5 years before and after, 04.86-08.97, was available on 26 feeding studies on 950 animals. One drinking study was excluded from the provided HCD since spontaneous tumour rates can be affected by diet. The criteria for same/species, supplier and testing facility were considered to be met.

Considering brain tumours, especially gliomas/glioblastomas, the testing laboratory had informed on changes in nomenclature in diagnostic terms over the time period. Thefore, these inconsistencies need to be taken into account in assessing the brain tumour findings of carcinogenicity study. Of note, the HCD of brain tumours cover time period of 04.86-11.98 and 1020 studies (Table 34).

**Table 34: HCD on brain tumours in 1020 studies.**

| M/F | Schwannoma | Meningioma | Glioma | Glioblastoma | Oligodendroglioma |
|---|---|---|---|---|---|
| **Males** | 1 | 5 | 3 | 1 | 3 |
| **Females** | 2 | 2 | 3* | - | 4 |

*includes 2 cases of astrocytomas

As described by Krinke et al. (2000) a diagnosis of "glioma" is sometimes used to present all gliomas and sometimes to additionally include astrocytomas and oligodendrogliomas. The general term of glioma is used for technical reasons (i.e due to autolysis) when a precise classification is not possible. Since the HCD provided by the Notifier has been compliled from data with varying classification criteria, in addition to category of glioma, a combination of brain tumours, glioma+glioblastoma and glioma+glioblastoma+oligodendroglioma, were used in assessing brain tumour findings in females reported in chronic toxicity and carcinogenicity studies.

**Table 35: Summary of neoplastic findings in male Wistar rats. Chronic toxicity and carcinogenicity studies.**

| Organ | Control | 290 ppm | 2316 ppm | 5790 ppm | HCD, range or mean |
|---|---|---|---|---|---|
| **Adrenal cortex** | | | | | |
| -adenoma | 1/69 (1.4%) | 2/32 | 1/30 | 2/70 (2.9%) | 0-12% |
| **Mammary gland** | | | | | |
| -fibroadenoma | - | - | - | 1/ 2 | 0-2% |
| -adenocarcinoma | - | - | - | 1/ 2 | 0-5% |
| **Thyroid gland** | | | | | |
| -C-cell adenoma | 4/70 (5.7%) | 0/18 | 3/17 | 7/70 (10%) | 0-20% |
| **Pancreas** | | | | | |
| -islet cell adenoma | 2/70 (2.9%) | 0/20 | 0/16 | 3/70 (4.3%) | 0-10% |
| -islet cell carcinoma | 0/70 | 2/20 | 0/16 | 0/70 | 0-5% |
| **Spleen** | | | | | |
| -hemangiosarcoma | 1/70 (1.4%) | 7/27 | 4/21 | 3/70 (4.3%) | 0-10% |
| **Urinary bladder** | | | | | |
| -urothelial papilloma | 1/70 (1.4%) | 0/21 | 2/19 | 3/70 (4.3%) | 0-5% |

| | | | | | |
|---|---|---|---|---|---|
| **Brain** | | | | | |
| -meningioma | 0/70 | 0/21 | 0/17 | 1/70 (1.4%) | 0.49% |
| -schwannoma | 0/70 | 0/21 | 0/17 | 1/70 (1.4%) | 0.1% |

**Table 36: Summary of neoplastic findings in female Wistar rats. Chronic toxicity and carcinogenicity studies.**

| Organ | Control | 290 ppm | 2316 ppm | 5790 ppm | HCD, range or mean |
|---|---|---|---|---|---|
| **Liver** | | | | | |
| -carcinoma | 0/70 | 1/70 | 2/70 | 3/70 (4.3%) | 0-5% |
| **Pancreas** | | | | | |
| -islet cell carcinoma | 0/70 | 2/21 | 0/19 | 0/69 | 0-2% |
| **Mammary gland** | | | | | |
| -adenocarcinoma | 4/67 (6.0%) | 3/24 | 5/25 | 8/70 (11.4%) | 0-25% |
| **Uterus** | | | | | |
| -adenocarcinoma | 0/70 | 1/33 | 1/33 | 2/70 (2.9%) | 0-5% |
| -stromal polyp | 2/70 (2.9%) | 1/33 | 4/33 | 4/70 (5.7%) | 0-18% |
| -hemangiosarcoma | 0/70 | 0/33 | 1/33 | 2/70 (2.9%) | 0-10% |
| **Brain** | | | | | |
| -glioblastoma | 0/70 | 0/32 | 0/32 | 2/70 (2.9%) | 0% |
| -glioma+glioblastoma | 1/70 (1.4%) | 0/32 | 0/32 | 2/70 (2.9%) | 0.67% |
| -glioma+glioblastoma+oligodendroglioma | 2/70 (2.9%) | 0/32 | 0/32 | 2/70 (2.9%) | 0.69% |

Males

Adenomas of adrenal cortex, thyroid C-cell adenomas and urinary papillomas showed elevated incidences when compared to concurrent control, but remained within the range of HCD and thus considered not related to treatment.

Mammary gland fibroadenomas and adenocarcinomas are not required organs for histopathological investigations in male rats according to OECD 451 guideline. In the present study one male was diagnosed with a fibroadenoma and one with an adenocarcinoma. According to HCD these rare male mammary gland tumours have ranges in incidencies of 0-2% and 0-5%, respectively, in Wistar rats. These tumours are considered unlikely to be treatment related.

The elevated numbers of pancreas islet adenomas at the high dose level and islet cell carcinomas at the low dose level are considered incidental since their incidences fall within the range of HCD.

The number of spleen hemangiosarcomas, 7/27, at the low dose level exceeds the HCD. However at the high dose level the incidence was lower (3/70) indicating a lack of dose-response relationship. The observed neoplasms at the lowest dose level are considered unlikely to be related to the treatment.

Regarding brain tumours, one case of meningioma was found among males of high dose group. In HCD the frequency of meningiomas seems to be low. According to Krinke et al. 2000 in studies using both the diagnosis of meningiomas and granular cell tumours, the incidences of lesions should be evaluated jointly. Considering one case of a granular cell tumour (see table) in male controls, the occurance of a meningioma in one male of high dose level can be interpreted as being spontaneous. One male of high dose group had a diagnosis of brain schwannoma. According to HCD these rare tumours have been detected in 1/1020 animals. Brain schwannoma in one male of high dose group may be considered incidental. Schwannomas in varied sites of control Wistar rats are reported to occur with an incidence of 1.51% (Poteracki and Walsh, 1998).

Females

In females of high dose group incidence of liver carcinoma, mammary carcinoma and uterine adenocarcinoma, stromal polyp and hemangioma was slightly increased as compared to concurrent control but within the range of HCD.

Two cases of islet cell carcinomas of pancreas were detected in females of low dose group (in control 0/70). the incidence of this lesion is above HCD. As dose-response relationship cannot be seen and no cases pancreatic adenomas of islet cells cases were not reported in females, the finding is considered incidental.

Two cases of glioblastomas were seen in females of high dose group and 0/70 in controls. According to Krinke et al. (2000) a combination of tumours with terms glioma, glioblastoma and oligodendroglioma can be used in carcinogenicity studies. This combination in controls and high dose males shows an equal incidence (2/70 in both). In the study Bomhard (1992) on spontaneous nervous system tumours of Wistar rats, incidence of glioblastomas in females was 2.0%. It can be concluded that the observed gliomas/glioblastomas in the rat carcinogenicity study are not related to mepiquat chloride treatment.

Combined oral toxicity and carcinogenicity study (Leuschner et al. 1979, non-guideline)

The study was conducted before mandatory GLP certification requirements and in accordance with in-house methodology. Groups of 105, 55, 50 and 35 male and female Sprague Dawley rats were administered mepiquat chloride in the diet for up 104 weeks at concentrations of 0, 100, 300, 1000, 3000 and 9000 ppm corresponding to achieved daily intakes of 0, 6, 18, 62, 186 and 684 mg/kg bw/day in males and 0, 7, 21, 72, 212 and 670 mg/kg bw/day in females. Groups of 5 male and 5 female animals for the control, 3000 ppm and 9000 ppm dose groups were designated for the interim-kill at 52 weeks. All animals, which died prior to scheduled sacrifice and those, which were killed at the termination of treatment were examined macroscopically and the weights of heart, liver, lungs, spleen, kidney, adrenal, thymus, pituitary, gonads, thyroid, brain were recorded. The following organs of experimental and control animals were investigated: heart, liver, lungs, spleen, kidney, adrenal gland, thymus, pituitary gland, gonads, trachea, thyroid, brain, prostate, uterus, stomach, duodenum, jejunum, ileum, colon, rectum, aorta, salivary gland, eye, urinary bladder, bone marrow, oesophagus, pancreas, lymph node, bone, mammary gland, skeletal muscle and peripheral nerve. The study lacks detailed clinical examinations. Other shortcomings concern the performance of hematology examinations.

**Results**

No significant effects on body weights were reported during the course of the study with the exception that terminal body weight was reduced in males (11%) and females (14 %) at 9000 ppm compared with controls. No significant effects on mortality was observed.

**Table 37: Body weights (g) and mortality rates (%).**

| Parameter | Control | 100 ppm | 300 ppm | 1000 ppm | 3000 ppm | 9000 ppm |
|---|---|---|---|---|---|---|
| **Males** | | | | | | |
| **Body weights (terminal)** | 564.2 | 585.6 | 583.3 | 570.2 | 569.8 | 500.8 (89%) |
| **Mortality** | 28 | 30 | 22 | 20 | 22 | 20 |
| **Females** | | | | | | |
| **Body weights (terminal)** | 372.8 | 379.6 | 369.1 | 384.2 | 372.6 | 320.1 (86%) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Mortality** | 19 | 10 | 18 | 18 | 22 | 20 |

In females at week 52, clinical chemistry analyses revealed a statistically significant increase (42%) in alkaline phosphatase level at 3000 ppm. Moreover, $\alpha_1$-globuline was statistically significantly (28%) increased at 100 ppm.

No statistically significant changes in haematology and urinalysis parameters were found. No treatment-related findings in microscopy and macroscopy analyses were reported.

In males, the absolute weight of adrenals were statistically significantly decreased among groups of 1000 ppm, 3000 ppm and 9000 ppm at study termination. The absolute weight of brain was statistically significantly decreased at the top dose. In females, organ weights revealed statistically significant reductions of the absolute weight of heart, liver and kidneys in females of the high dose group. A slight reduction in relative adrenal weights in both males and females was observed.

**Table 38: Absolute organ weights (g).**

| Organ | Control | 100 ppm | 300 ppm | 1000 ppm | 3000 ppm | 9000 ppm |
|---|---|---|---|---|---|---|
| **Males** | | | | | | |
| **Adrenals** | 0.034 | 0.034 | 0.033 | 0.028* (82%) | 0.029* (83%) | 0.026* (76%) |
| **Brain** | 2.31 | 2.28 | 2.29 | 2.28 | 2.24 | 2.08* (90%) |
| **Females** | | | | | | |
| **Heart** | 1.29 | 1.27 | 1.22 | 1.25 | 1.25 | 1.11* (86%) |
| **Liver** | 12.7 | 12.7 | 12.2 | 12.8 | 12.1 | 11.4* (90%) |
| **Kidney** | 1.24 | 1.25 | 1.25 | 1.21 | 1.22 | 1.13* (91%) |

*$p<0.01$, values in parentheses are % of control- values

**Table 39: Relative adrenal weights.**

| Control | 100 ppm | 300 ppm | 1000 ppm | 3000 ppm | 9000 ppm |
|---|---|---|---|---|---|
| **Males** | | | | | |
| 0.06/0.06 | 0.058 | 0.056 (93%) | 0.08 (80%) | 0.05 (84%) | 0.052 (87%) |
| **Females** | | | | | |
| 0.118/0.112 | 0.104 (91%) | 0.106 | 0.101 (88%) | 0.105/0.105 (92%) | 0.119 |

Values in parentheses are % of control- values

**Table 40: Overview on tumour findings in male/female rats.**

| Dose group (ppm) | 0 | 100 | 300 | 1000 | 3000 | 9000 |
|---|---|---|---|---|---|---|
| **No. of examined animals (m/f)** | 100/100 | 50/50 | 50/50 | 50/50 | 50/50 | 30/30 |
| **Benign+ malignant tumours (%)** | 54/33 | 50/32 | 52/36 | 46/38 | 54/34 | 43/30 |
| **Tumour bearing rats (%)** | 49/32 | 46/30 | 42/36 | 44/36 | 46/34 | 46.7/30 |

No increases in numbers of benign nor malignant tumours were found in treated animal groups as compared to controls. Moreover, in individual organs no treatment-related changes in the incidences of tumours were observed. In conclusion, the non-guideline carcinogenicity study conducted in Sprague Dawley rats did not provide evidence of carcinogenic potential of mepiquat chloride.

**Mice**

Carcinogenicity study. B6C3F1/ClrBr mice

The study was conducted in accordance with the GLP provisions and principles and performed mainly according to OECD 451 (2009). Groups of 50 male and 50 female B6C3F1/CrlBr mice were administered mepiquat chloride (Purity: 58 %) in the diet for up to 104 weeks at concentrations of 0 (control), 500, 2000, or 7500 ppm in relation to a.i. (corresponding to achieved daily intakes of 0, 74, 297 or 1140 mg/kg bw/day in males and 0, 85, 328, or 1348 mg/kg bw/day in females). Satellite groups of 10 male and 10 female mice were similarly treated for 12 months before termination. The achieved dietary intakes for the satellite group over the same period was 0, 83, 314, and 1249 mg/kg bw/day in males and 0, 95, 414 and 1607 mg/kg bw/day in females.

Feed consumption and body weight were determined weekly for 14 weeks and every four weeks thereafter. The general state of animal health and mortality was checked daily; moreover, comprehensive clinical examinations of the animals were performed once a week. Blood was collected for differential blood smears of the animals of the satellite groups (day 369) and at study termination from all surviving animals of the main groups. Only the blood smears of the control and the high dose groups were evaluated. Furthermore, blood smears were prepared and evaluated from all animals killed in extremis during the study.

All animals were subjected to complete gross pathological examinations. The weights of selected organs brain, liver, kidneys, adrenal glands, testes) were determined. Gross lesions were examined for all animals affected.

Histopathological examinations were carried out on all animals of control and high dose groups, in addition lungs, liver, kidneys, adrenal glands and preputial glands were examined also from animals on low and mid dose groups. Animals that died intercurrently or were sacrificed in a moribund state were investigated histopathologically as outlined for the control animals.

Body weights of males in the main group were slightly decreased at the end of the study.

Survival was >80% for all groups at termination. Survival in satellite group was 100% among both males and females at all dose levels.

**Table 41: Body weight (g) and mortality (%) -main group.**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **Dose ppm** | **0** | **500** | **2000** | **7500** | **Control** | **500** | **2000** | **7500** |
| **Body weight** | 38.0 | 36.7 | 36.9 | 35.6 | 35.9 | 35.0 | 35.9 | 35.6 |
| **Mortality** | 16 | 4 | 4 | 14 | 18 | 12 | 18 | 16 |

In the differential blood count the number of monocytes was increased in males of satellite and termination groups compared to control. On the contrary, in females levels of monocytes were decreased at the same time points (day 369 and termination).

**Table 42: Differential blood count (day 369= satellite group, termination= main group).**

| **Parameter** | **Males** | | **Females** | |
|---|---|---|---|---|
| | **Control** | **7500 ppm** | **Control** | **7500 ppm** |
| **EOS (%)** | | | | |
| -day 369 | 1.2 | 2.80 (136%) | 2.00 | 1.2 (60%) |
| -termination | 1.6 | 1.49 | 1.32 | 1.6 (121%) |
| **BAND (%)** | | | | |
| -day 369 | 0.2 | 0.3 (150%) | 0.2 | 0.1 (50%) |
| -termination | 0.38 | 0.42 (111%) | 0.29 | 0.26 |
| **POLY (%)** | | | | |
| -day 369 | 16.30 | 14.30 (88%) | 14.10 | 15.90 (113%) |
| -termination | 22.36 | 22.60 | 21.95 | 16.88 (77%) |
| **MONO (%)** | | | | |
| -day 369 | 3.60 | 4.00 (111%) | 7.30 | 4.90 (67%) |
| -termination | 4.90 | 7.28 (149%) | 9.22 | 8.24 (89%) |
| **Changes in nucleus of lymphocytes, > 7** | | | | |
| -termination | 2/42 | 7/43 | 10/41 | 3/42 |
| **Juvenile lymphocytes, >2** | | | | |
| -termination | - | - | 3/41 | 9/42 |

In grosspathological examination the main finding was an elevated incidence of enlarged preputial glands in males of all dose groups, an increase being dose related.

**Table 43: Incidence of gross lesions – main groups.**

| Organ, findings | Control | 500 ppm | 2000 ppm | 7500 ppm |
|---|---|---|---|---|
| **Males** | | | | |
| Lungs, mass | 2 | 2 | 5 | 5 |
| Preputial glands, enlarged | 6 | 10 | 11 | 13 |
| Forestomach, focus | - | 5 | 2 | 4 |
| Glandular stomach, focus | - | 1 | 1 | 3 |
| **Females** | | | | |
| Liver, focus | 3 | 9 | 6 | 4 |
| Lungs, mass | 1 | 1 | 2 | 2 |
| Forestomach, focus | 1 | 1 | - | 3 |

In histopathological examination of organs and tissue vacuolization of kidneys and tubular hyperplasia in males were slightly increased at 2000 and 7500 ppm. Occurrance of spleen hyperplasia and hyperplasia in pancreas (islet) was elevated among females at 7500 ppm.

**Table 44: Microscopic findings – main groups.**

| Organ, findings | Control | 500 ppm | 2000 ppm | 7500 ppm |
|---|---|---|---|---|
| **Males** | | | | |
| **Kidneys** | | | | |
| -vacuolization | 22 | 24 | 35 | 34 |
| -hyperplasia, tubular | 25 | 27 | 35 | 30 |
| **Mesenteric lymph n.** | | | | |
| -hyperplasia, lymph | 3 | 5 | 4 | 5 |
| -hyperemia/blood aspr | 9 | 7 | 9 | 13 |
| **Pancreas** | | | | |
| -hyperplasia, islet | 7 | 1 | - | 10 |
| **Females** | | | | |
| **Kidneys** | | | | |
| -hyperplasia, tubular | 1 | 4 | 4 | 2 |
| **Spleen** | | | | |
| -hyperplasia, lymph | 3 | 4 | 5 | 6 |
| -hemosiderin storage | 1 | - | - | 3 |
| **Mesenteric lymph nodes** | | | | |
| -hyperplasia, lymph | - | - | 1 | 2 |
| **Iliac lymph nodes** | | | | |
| -hyperplasia, lymph | 2 | 4 | 2 | 7 |
| **Uterus** | | | | |
| -hydrometra | 3 | 7 | 9 | 5 |
| **Pancreas** | | | | |
| -hyperplasia, islet | 1 | - | 1 | 5 |

Overall, no difference in the incidence of benign and malignant neoplasms between control and test groups was found.

**Table 45: Microscopic findings – main groups.**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **Dose (ppm)** | **0** | **500** | **2000** | **7500** | **0** | **500** | **2000** | **7500** |
| Mice with neoplasms | 58 | 36 | 44 | 46 | 50 | 50 | 42 | 34 |
| Benign neoplasms | 44 | 22 | 36 | 26 | 24 | 32 | 22 | 12 |
| Malignant neoplasms | 32 | 16 | 22 | 28 | 34 | 34 | 26 | 26 |

**Table 46: Incidence of lung and liver tumours.**

| Organ | Control | 500 ppm | 2000 ppm | 7500 ppm | HCD* |
|---|---|---|---|---|---|
| **Males** | | | | | |
| **Lungs** | | | | | |
| -adenocarcinoma | 4% | 4% | 2% | 8% | 0-14% |
| -adenoma, br-alv. | 12% | 6% | 14% | 14% | 0-10% |
| -combined | 16% | 10% | 16% | 22% | 0-24% |
| **Females** | | | | | |
| **Lungs** | | | | | |
| -adenocarcinoma | 2% | 2% | - | 4% | 0-2% |
| -adenoma, br-alv. | 2% | 6% | 2% | 2% | 0-6% |
| -combined | 4% | 8% | 2% | 6% | 0-8% |
| **Liver** | | | | | |
| -hepatocell. carcinoma | 2% | - | 6% | 6% | 2-6% |
| -hepatocell. adenoma | 8% | 8% | 8% | 4% | 6-16% |

*) Appropriate HCD covering a five-year period before the study was available from two studies consisting of a total of 100 mice of same strain and supplier.

In lungs of males and lungs and liver of females slight, statistically insignificant, elevations in incidences of tumours at the top doses were detected when compared to concurrent controls.

In males of high-dose group incidence of lung adenocarcinoma and adenoma was elevated with frequencies 4/50 (8%) and 7/50 (14%), respectively as compared to concurrent control animals. However, the incidences do not show dose-response and were within the range of HCD, when incidencies of lung adenomacarcinomas and adenomas were combined.

In females, lung adenocarcinomas were detected in 2/50 (4%) at the highest dose. The frequency exceeds HCD. However, this elevation shows no dose-response, and is thus considered unrelated to the treatment. In the high-dose group the incidence of hepatocellular carcinoma 3/50 (6%) was higher than in the control group. The incidence was within the range of HCD.

**Carcinogenicity study. NMRI mice**

In a study (1979), groups of 100 (control) or 50 (test groups) male and female NMRI mice were administered mepiquat chloride in the diet at concentrations of 0 (control), 100, 300, 1000, or 3000 ppm of 0, 16, 48.9, 169.4 and 513.5 mg/kg bw/day in males and 0, 21.7, 65.3, 226.1 and 689.4 mg/kg bw/day in females. Haematology, clinical chemistry and urinalysis investigations were carried out on 10 animals/sex/dose group at 26, 52, and 104 weeks after the start of treatment.

All animals which died prior to scheduled sacrifice and those which were killed at the termination of treatment were subjected to a full autopsy. Histopathological examinations were performed of organs/tissues mainly in accordance with current guidelines with the exception of gall bladder, caecum, epididymis, cervix, coagulating gland, parathyroid, seminal vesicle, skin and spinal cord which were not investigated.

There were deviations from OECD guideline. Detailed clinical examinations were not made. Performance of all hematology and clinical chemistry examinations were not conducted, performed less often and with less animals than according to guideline. The weights of adrenals, epididymides, thyroid and uterus were not measured. Caecum, cervix, coagulating gland, epididymis, gall bladder, parathyroid, seminal vesicle, skin and spinal cord were not examined histopathologically. The study was conducted before mandatory GLP requirements.

No significant effects on body weights and no effect on mortality were observed at the end of the study.

**Table 47: Body weight and mortality.**

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dose (ppm)** | **0** | **100** | **300** | **1000** | **3000** | **0** | **100** | **300** | **1000** | **3000** |
| **Body weight (g)** | 42.3 | 43.5 | 42.5 | 40.8 | 42.2 | 35.3 | 35.9 | 37.3 | 36.0 | 35.0 |
| **Mortality (%)** | 49 | 48 | 48 | 54 | 50 | 61 | 64 | 62 | 60 | 60 |

In clinical chemistry and urine analyses no consistent treatment-related findings were observed. In males ncreases in leucocyte counts at all doses from week 26 to 104 were found. The change was statistically insignificant except in at week 52 in low dose group with an increase of 30% as compared to control animals.

**Table 48: Body weight and mortality.**

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dose (ppm)** | **0** | **100** | **300** | **1000** | **3000** | **0** | **100** | **300** | **1000** | **3000** |
| **week 26** | 8.6 | 8.8 | 9.6 (112%) | 10.2 (119%) | 10.2 (119%) | 8.8 | 9.3 | 9.5 | 9.4 | 8.9 |
| **week 52** | 7.9 | 10.3* (130%) | 9.5 (120) | 9.8 (124%) | 10.1 (128%) | 9.6 | 8.8 | 10.4 | 9.9 | 9.4 |
| **week 104** | 6.9 | 8.0 | 8.0 (116%) | 8.7 (126%) | 9.1 (132%) | 8.1 | 8.6 | 8.8 | 8.9 | 8.9 |

Changes in relative and absolute weight of spleen were observed in all treated males. A reduction of 79-89% in 100 ppm, 300 ppm and 1000 ppm groups and an increase of 26-27% at the dose level of 3000 ppm were reported. In females relative organ weight of thymus was decreased by 27% at the top dose.

**Table 49: Spleen and thymus weights.**

| Organ | Control | 100 ppm | 300 ppm | 1000 ppm | 3000 ppm |
|---|---|---|---|---|---|
| **Males** | | | | | |
| **Spleen** | | | | | |
| -relative weight | 4.49 | 3.91 (87%) | 3.53 (79%) | 3.92 (87%) | 5.69 (127%) |
| -absolute weight | 0.19 | 0.17 (89%) | 0.15 (79%) | 0.16 (84%) | 0.24 (126%) |
| **Females** | | | | | |
| **Spleen** | | | | | |
| -relative weight | 7.16 | 8.31 (116%) | 6.72 | 6.74 | 8.53 (119%) |
| **Thymus** | | | | | |
| -relative weight | 1.10 | 1.07 | 1.29 (117%) | 1.08 | 0.80 (73%) |

Benign and malignant neoplasms were found in animals of control and treatment groups at equal frequencies.

**Table 50: Frequencies (%) of benign and malignant tumours.**

| Group (ppm) | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **100** | **300** | **1000** | **3000** | **0** | **100** | **300** | **1000** | **3000** |
| Mice with tumours | 67 | 52 | 46 | 36 | 38 | 71 | 70 | 54 | 72 | 64 |
| Benign tumours | 18 | 20 | 20 | 12 | 10 | 15 | 24 | 16 | 32 | 36 |
| Malignant tumours | 62 | 42 | 34 | 28 | 34 | 69 | 76 | 54 | 74 | 48 |

Frequencies of lymphomas, pituitary adenomas, uterine leiomyomas and necrotic tumours of ovary were increased as compared to concurrent controls. All elevations were statistically insignificant.

**Table 51: Incidencies of neoplasias.**

| Tumour/neoplasm | Males (ppm) | | | | | Females (ppm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **100** | **300** | **1000** | **3000** | **0** | **100** | **300** | **1000** | **3000** |
| Lymphoma (%) | 1 | 2 | 4 | 2 | 4 | 1 | 2 | 3 | 0 | 8 |
| Adenoma: pituitary (%) | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 2 | 4 |
| Leiomyoma: uterus (%) | - | - | - | - | - | 0 | 0 | 2 | 0 | 4 |
| Necrotic tumour: ovary (%) | - | - | - | - | - | 0 | 0 | 0 | 0 | 4 |

An increase in the incidence of lymphomas was observed both in males and females at all dose levels except at 1000 ppm in females. Neither in males nor in females a dose-response relationship is observed. No HCD is available for the study conducted in 1979. In a publication by Bomhard and Mohr (1989) spontaneous tumours in untreated NMRI mice from twelve carcinogenicity studies from two different breeders between 1974 and 1979 were compiled. Tumours located in lymphoreticular system were found in 28% of animals indicating that this type of neoplasm is relatively common in NMRI mice. Therefore, lymphomas found in this study are considered incidental.

In females, pituitary adenomas were found in 4% of females (1% in controls). Average frequency of pituitary adenomas in the study of Bomhard and Mohr (1989) was 2%. As no dose-response relationship cannot be seen, the occurrance of these tumours is regarded as a spontaneous event.

Uterine leiomyomas were detected with incidences of 4% at the top dose and 2% at 300 ppm. No dose-response cannot be observed.

There is some concern related to the incidence (4%) of necrotic tumours in the ovary at the highest dose (0% in control). Of note, the exact type of tumour, is not indicated in the study report, obviously due to difficulties in the pathological diagnosis. The average frequency in the category of ovarian tumours is 21% in NMRI mice according to Bomhard and Mohr (1989). Based on the relatively high incidence of ovarian tumours in this mouse strain, the two (4%) necrotic ovarian tumours found in the study with mepiquat chloride are regarded as incidental findings.

### 10.9.1 Comparison with the CLP criteria

Based on available *in vitro* and *in vivo* studies no evidence on genotoxicity of mepiquat chloride is observed.

There is no evidence of mepiquat chloride having caused cancer in humans.

Chronic toxicity and carcinogenicity studies were conducted in rats and mice. The tumours observed in treated animals were statistically insignificant for neoplastic findings showing elevated incidences in treated animals. Comparisions with concurrent control and assessments of dose-response relationship were made for neoplastic findings showing elevated incidences in treated animals. Morever, HCD was used when available, in order to consider the presence of spontaneous tumours. In the absence of applicable HCD from the study laboratory, relevant publications were used as supplemental source of information.

Inconsistencies in diagnostic criteria and challenges in classification of brain gliomas/glioblastomas in rat carcinogenicity study and ovarian tumours in mouse (NMRI) carcinogenicity study were noted. Taken into account this it is concluded that these findings are regarded as non-treatment related.

Overall, the elevations in incidences of some tumours discussed in this report are considered to result from spontaneous carcinogenic events without a link to mepiquat chloride exposure.

According to the CLP criteria no classification as a carcinogen category 1 or 2 is required.

### 10.9.2 Conclusion on classification and labelling for carcinogenicity

No classification for carcinogenicity according to the CLP Regulation is proposed.

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

The carcinogenic potential of mepiquat chloride has been investigated in rats and mice. The DS concluded that there is no evidence of a treatment-related increase in tumours and proposed no classification for carcinogenicity.

### Comments received during consultation

Comments were received from an MSCA and a manufacturer. Both supported no classification.

### Assessment and comparison with the classification criteria

*2-year study in rats (1994)*

In this OECD guideline- and GLP-compliant study Wistar rats (70/sex/group) were administered mepiquat chloride at dietary levels up to 5790 ppm (ca. 320 mg/kg bw/d). Body weight was reduced by about 10-20% compared to controls at the top dose, survival was not affected. No macroscopic or histopathological evidence of carcinogenicity or systemic toxicity was found in this study according to the study authors.

The DS discussed incidence of several tumours. Incidences of selected tumour types as given in the original study reports are provided in the table below. In the low- and mid-dose a complete histopathology was performed only in animals that died or were sacrificed in extremis, whereas in the survivors only lungs, liver, kidneys and organs with gross lesions were examined microscopically. Incidences at low- and mid-dose are not shown for organs not examined in all animals as such incidences are not representative of the whole group.

**2-year rat study (1994): incidence of selected neoplastic and preneoplastic findings** (out of 67-70 animals/sex/group)

| | **Males** | | | | **Females** | | | |
|---|---|---|---|---|---|---|---|---|
| **Dose (ppm)** | **0** | **290** | **2320** | **5790** | **0** | **290** | **2320** | **5790** |
| Dose (mg/kg bw/d) | 0 | 13 | 110 | 270 | 0 | 18 | 140 | 370 |
| Brain: granular cell tumour, meningioma | 5 (7.1%) | | | 2 (2.9%) | 1 (1.4%) | | | 1 (1.4%) |
| Brain: glioma, glioblastoma, oligodendroma | 0 (0%) | | | 1 (1.4%) | 2 (2.9%) | | | 2 (2.9%) |
| Brain: schwannoma | 0 (0%) | | | 1 (1.4%) | 0 (0%) | | | 0 (0%) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Liver: adenoma | 0 (0%) | 2 (2.9%) | 0 (0%) | 2 (2.9%) | 1 (1.4%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Liver: carcinoma | 6 (8.6%) | 3 (4.3%) | 4 (5.7%) | 5 (7.1%) | 0 (0%) | 3 (4.3%) | 2 (2.9%) | 3 (4.3%) |
| Urinary bladder: urothelial papilloma | 1 (1.4%) | | | 3 (4.3%) | 0 (0%) | | | 0 (0%) |
| Urinary bladder: urothelial hyperplasia | 4 (5.7%) | | | 5 (7.1%) | 0 (0%) | | | 0 (0%) |
| Uterus: adenoma | | | | | 1 (1.4%) | | | 0 (0%) |
| Uterus: adenocarcinoma | | | | | 0 (0%) | | | 2 (2.9%) |
| Uterus: squamous carcinoma | | | | | 0 (0%) | | | 1 (1.4%) |
| Uterus: stromal sarcoma | | | | | 2 (2.9%) | | | 1 (1.4%) |
| Uterus: stromal polyp | | | | | 4 (5.7%) | | | 4 (5.7%) |
| Uterus: hemangiosarcoma | | | | | 0 (0%) | | | 2 (2.9%) |
| Uterus: leiomyoma | | | | | 0 (0%) | | | 2 (2.9%) |
| Mammary gland: adenocarcinoma | | | | | 4 (6.0%) | | | 8 (11.4%) |

None of the differences between the control and treated groups is statistically significant in Fisher test. Historical control incidences for uterine and mammary gland tumours (28-29 studies within 5 years of the current study, the same laboratory, the same source of animals; for some other tumour types HCD was also available but not considered needed since the incidence at the top dose was comparable to the incidence in concurrent controls) are as follows:

- Uterus, adenocarcinoma: occurred in 6 studies, incidence 1 animal out of 50 or 20
- Uterus, hemangiosarcoma: occurred in 5 studies, incidence 1 animal out of 50 or 20
- Mammary gland, adenocarcinoma: range 0-25%, mean 9%; incidence at the top dose in the current study is 11%

RAC agrees with the DS that the results of this study do not show evidence of a treatment-related neoplastic effect.

2-year study in rats (1979)

In this pre-guideline study Sprague-Dawley rats were administered mepiquat chloride at dietary concentrations up to 9000 ppm (equivalent to ca. 680 mg/kg bw/d). Body weight at the top dose was reduced by ca. 10% compared to controls. No increase in tumours was found in this study.

2-year study in mice (1994)

In this OECD guideline- and GLP-compliant study B6C3F1 mice (50/sex/group, plus 10/sex/group for interim sacrifice) were administered mepiquat chloride at dietary levels up to 7500 ppm (ca. 1200 mg/kg bw/d). There was no general toxicity besides a slight body weight reduction in top dose males. No evidence of a neoplastic potential was found in this study.

2-year study in mice (1979)

In this pre-guideline study NMRI mice (50/sex/group, control 100/sex) were administered mepiquat chloride at dietary levels up to 3000 ppm (equivalent to ca. 600 mg/kg bw/d). There was no general toxicity besides a slight body weight reduction in top dose males. No evidence of a neoplastic potential was observed in this study according to the study authors.

The DS discussed several tumour types, incidences are shown in the table below. There is no statistically significant difference in Fisher test besides lymphoma. Differentiation between leukaemia and lymphoma was very difficult according to the study report and there is no longer an increase when these two neoplasms are combined. RAC agrees with the DS that this study does not provide evidence of a treatment-related neoplastic effect.

| **2-year mouse study (1979): incidence of selected neoplastic findings** | | | | | |
|---|---|---|---|---|---|
| **Dose (ppm)** | **0** | **100** | **300** | **1000** | **3000** |
| Dose (mg/kg bw/d) | 0 | 19 | 57 | 200 | 600 |
| No. of examined animals per sex | 100 | 50 | 50 | 50 | 50 |
| Leukaemia – males | 54 (54%) | 15 (30%) | 8 (16%) | 12 (24%) | 13 (26%) |
| Lymphoma – males | 1 (1%) | 1 (2%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Leukaemia – females | 59 (59%) | 28 (56%) | 20 (40%) | 33 (66%) | 14 (28%) |
| Lymphoma – females | 1 (1%) | 1 (2%) | 2 (4%) | 0 (0%) | 4 (8%) |
| Pituitary: adenoma – males | 0 (0%) | 0 (0%) | 1 (2%) | 0 (0%) | 0 (0%) |
| Pituitary: adenoma – females | 1 (1%) | 0 (0%) | 0 (0%) | 1 (2%) | 2 (4%) |
| Ovary: granulosa thecoma | 5 (5%) | 1 (2%) | 0 (0%) | 6 (12%) | 3 (6%) |
| Ovary: fibroma | 0 (0%) | 0 (0%) | 0 (0%) | 1 (2%) | 0 (0%) |
| Ovary: necrotic tumour (type not specified) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 2 (4%) |
| Uterus: myoma, leiomyoma | 2 (2%) | 3 (6%) | 1 (2%) | 0 (0%) | 2 (4%) |

| | | | | | |
|---|---|---|---|---|---|
| Uterus: leiomyosarcoma | 0 (0%) | 0 (0%) | 1 (2%) | 0 (0%) | 0 (0%) |

***Genotoxicity***

The mutagenicity hazard class was not open for the consultation and data was presented only as background information for carcinogenicity assessment in the CLH report. All available genotoxicity studies (including a valid Ames test, a valid *in vitro* micronucleus test and a valid *in vivo* micronucleus test) are negative. A brief overview of genotoxicity studies can be found under 'supplemental information'.

***Conclusion on classification***

There was no evidence of a treatment-related increase in neoplastic findings in the available studies. Therefore, RAC agrees with the DS's proposal of **no classification for carcinogenicity.**

## Supplemental information - In depth analyses by RAC

***Overview of genotoxicity studies***

The following table provides a brief overview of the available genotoxicity studies with mepiquat chloride.

| **Genotoxicity studies** | | | |
|---|---|---|---|
| **Type of study** | **Year** | **Result** | **Remarks** |
| ***In vitro*** | | | |
| Ames | 1979 | Negative | TA102 or *E.coli* WP2 not tested; top dose 2500 µg/plate without cytotoxicity |
| Ames | 1990 | Negative | |
| HPRT | 2002 | Negative | |
| Chromosomal aberrations | 1987 | Negative | Experimental design (e.g. exposure times) not in line with the current version of OECD TG 473 |
| Micronucleus | 2019 | Negative | |
| UDS | 1987 | Negative | |
| DNA repair test on *Bacillus subtilis* | 1990 | Negative | |
| ***In vivo*** | | | |
| Micronucleus (bone marrow; mouse, oral gavage) | 2002 | Negative | Mortality at the top dose, clinical signs of toxicity<br>Bone marrow exposure in NMRI mice demonstrated in a |

| | | | |
|---|---|---|---|
| | | | separate toxicokinetic study (2016) |
| Dominant lethal assay (mouse, dietary) | 1977 | Negative | |

## 10.10 Reproductive toxicity

**Two-generation study**

**Test guideline and GLP**

The study was conducted in accordance with the GLP-provisions and mainly according to OECD guideline 416 (2001). According to OECD 416, for the dietary studies the dose interval should not be more than 3 fold. This is not met between mid and high dose groups. It was not reported if more detailed clinical observation/examination of animals was done on weekly basis. At necropsy, the weight of uterus, ovaries, prostate, seminal vesicles, brain, spleen, pituitary, thyroid and adrenal glands were not measured on parental animals. Sperm counts, oestrus cycle, sexual maturation as according to OECD 416 were not measured. It was not reported if detailed testicular histopathology and qualitative/quantitative evaluation of primordial follicles were conducted. Organ weights of pups were not determined at necropsy. According to OECD 416 grossly abnormal tissue and target organs from all pups with external abnormalities or clinical signs, as well as from at least one randomly selected pup/sex/litter from both the F1 and F2 generation which have not been selected for mating, shall be fixed and stored in a suitable medium for histopathological examination. Full histopathological characterisation of preserved tissue should be performed with special emphasis on the organs of the reproductive system. In this study no histopathology on pups was reported. Historical control information was provided from years 1987 to 1992.

**Materials and methods**

In this multigeneration reproduction study (1993), with two matings for the first generation and one for the second generation, mepiquat chloride (batch no.: WW 262/ CP 1490, purity: 57.9 % w/w mepiquat chloride and 44.3% w/w water) was administered to groups of 25 male and 25 female Wistar parental rats (F0) in the diet at nominal concentrations of 0 (control), 500, 1500 or 5000 ppm (referring to active ingredient; 0, 864, 2591 and 8636 ppm test substance) continuously throughout the phases of the study (F0: with the exception of infertile animals and those which were taken for urinalyses). Concentration control of mixtures of food and test substance was determined at the beginning of the study and then approximately three monthly intervals.

At least 70 days after the beginning of substance administration F0 animals were mated at ratio of 1:1 to produce the F1a litter. At least 10 days after the last weaning of the F1a generation pups, the F0 parental animals were mated again to produce the F1b litter. After the F1b generation pups had been weaned, the F0 generation parental animals were sacrificed, together with the animals which were taken for the reevaluation of their fertility.

Groups of 25 males and females selected from the F1a pups, to form F1 parental generation, were offered the same concentrations in the feed post weaning continuously throughout the phases of the study (F1: with the exception of infertile animals and those which were taken for urinalyses). At least 98 days after formation of the F1 parental animals, F1 animals were mated at ratio of 1:1 to produce the F2 litter. Some weeks after the F2 generation pups had been weaned, F1 parental animals were sacrificed. For both F0 and F1 parental animals, the day on which sperm was detected in vaginal smear, was denoted day 0.

The F1a, F1b and F2 generation pups were standardized. On day 4 p.p. the individual litters were in general standardized so that each litter contained 4 male and 4 female pups. If not possible, 8 pups per litter were present for further rearing. With the exception of the F1a pups chosen for F1 parental animals, all pups were sacrificed after standardization or weaning and examined macroscopically. Thereafter, stillborn pups, those that died during the rearing period, pups which were culled on day 4 p.p. or "surplus" pups which showed any remarkable findings during rearing or abnormalities in the macroscopic assessment were examined additionally using appropriate methods.

If F0 or F1 parental animal had not produced any offspring, these animals were again mated for not more than 3 weeks with one fertile animal of the control group. The relevant male animals were sacrificed together with the majority of the other animals as scheduled. The relevant F0 females were sacrificed before littering or about 10 days after the last mating. Those F0 and F1 parental animals whose fertility had to be reevaluated were offered test substance free diet during matings. In the remaining time, these animals were offered food with test substance.

The food consumption was determined for males and females during the first 10 (F0 parent) or 14 (F1 parent) test week. For males, food consumption was not determined any longer after $10^{th}$ or the $14^{th}$ week. For females, food consumption during pregnany, and lactation period was determined, however, between days 14 and 21 after parturition food consumption was not determined. For females without positive evidence of sperm, without litters or for those animals whose fertility had to be reevaluated either during the additional matings or the interval until they were sacrificed food consumption was not determined. Water consumption was determined during the first 10 (F0 parent) or 14 (F1 parent) test week.

Body weights of parental animals were, in general, determined once a week. Some exceptions were applied to female parental animals. Clinical observations, including nesting, littering and lactation behavior, were done daily on parental animals. Mortality was checked daily. The examination of the neural function was carried out in all F0 and F1 parental animals (some days before the beginning of the mating period for F1a or F2 litters), in all F0 and F1 dams (during the lactation periods of the F1a, F1b and F2 pups) and in all F0 and F1 dams after weaning of the F1a, F1b and F2 pups and at about the same time in all F0 and F1 males.

Male/female mating index (%), male/female fertility index (%), gestation index and live birth index (%) were calculated.

All F1a, F1b and F2 pups were examined to determine the total number of pups and the number of liveborn and stillborn members of each litter. Mortality was checked daily. The number and percentage of dead pups and live pups/litter were calculated. Also viability and lactation indices (%) and sex ratio were calculated. Body weigh data of pups were determined and clinical symptoms examined daily. All surviving pups were tested for gripping reflex, hearing, and pupillary reflex.

The following examinations were intended to be carried out in 12 animals per test group and sex of the F0 and F1 parent: hematological examinations (leukocytes, erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, differential blood count, reticulocytes), clotting analyses (thromboplastin time), clinicochemical examinations (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum cholinesterase, erythrocyte cholinesterase, brain cholinesterase, serum-γ-glutamyltransferase, sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, abumin, globulins, triglycerides, cholesterol, magnesium) and urinalyses (volume, color, turbidity, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, specific gravity, sediment).

All F0 and F1 parental animals were assessed by gross pathology including weight determinations of several organs (liver, kidneys, epididymides, testes). 12 organs and all gross lesions were fixed in formaldehyde solution and followed by histotechnical processing. Histopathological assessment was done for all animals in high dose and control group. Liver and kidneys were examined also for all animals in low and mid dose groups, otherwise, only infertile animals in low and mid dose groups were examined. Gross lesions were examined on all affected animals.

**Results** F0/F1 parental animals:

The observed effects were similar between F0 and F1 parental animals. Food consumption was reduced, especially during lactation of F1a, F1b and F2 pups in high dose group. Body weight gain was impared in high dose group, being negative during lactation of F1a, F1b and F2 pups. During lactation of F1a, body weight was reduced also at 1500 ppm and during lactation of F2 pups in all dose groups. Tremor and hypersensitivity were observed in most high dose F0 and F1 dams. Grip strength of the forelimbs was reduced in high dose F0 dams, F1 males and F1 females. The grip strength values of the hindlimbs were decreased in the males and females in high dose group. The absolute weights of livers of F0 and F1 animals and kidneys of F1 animals were decreased in high dose group. The lipid storage in livers of high dose F0 females and F1 males and females was diminished clearly.

Based on the reduced body weight in females at 1500 ppm dose group during lactation of F2 pups, NOAEL of parental animals is 500 ppm (52 mg/kg bw/day).

## Adverse effects on sexual function and fertility

The gestation time was slightly, but statistically significantly, shorter for the F1a and F1b litter for high dose F0 dams. For F1 dams, the mean duration until sperm was detected was slightly extended, without statistical significance, for the high dose animals. The total number of F1a litter, total F1a pups delivered and F1a liveborn pups were reduced in the high dose group compared to control. The reduction in total number of litters, total pups delivered and liveborn pups were observed also at high dose F2 generation.

Based on these findings, NOAEL for reproductive effects is 1500 ppm (155 mg/kg bw/day). It should be noted however, that all the parameters according to OECD 416 were not determined.

## Adverse effects on development

Mean body weight gains of F1a, F1b and F2 pups were reduced in the high dose groups. Number of F1a, F1b and F2 pups reaching the criteria of auditory canal opening and eye opening and gripping reflex of F1b pups was decreased in the high dose group. A high number of pups in F1a high dose group died or were cannibalized and both the F1a viability index and lactation index are impaired in high dose group compared to control. The total number of delivered pups was decreased in F2 high dose group.

NOAEL for offspring is therefore 1500 ppm (155 mg/kg bw/day). Anophthalmia and hydrocephaly were observed in one pup at mid dose. It should be noted that organ weights were not recorded at necropsy and histopathology are missing. Tables below summarise developmental findings in F1- and F2-generations. In the historical control data which listed data from 9647 pups, one case of hydrocephalus was reported. Anophtalmia was reported in 5 pups (0.01%).

Table 52: Selected pup physical development and reflex data (F1).

| Parameter | F1a | | | | F1b | | | |
|---|---|---|---|---|---|---|---|---|
| | **0 ppm** | **500 ppm** | **1500 ppm** | **5000 ppm** | **0 ppm** | **500 ppm** | **1500 ppm** | **5000 ppm** |
| Pinna unfolding (N, pups reaching criteria) | 310 | 286 | 330 | 157* | 329 | 321* | 330* | 236* |
| Auditory canal opening (N, pups reaching criteria) | 188 | 174[1] | 191 | 88* | 194 | 186 | 189 | 141* |
| Eye opening (N, pups reaching criteria) | 190 | 182 | 187 | 102* | 189 | 191* | 189[1] | 141* |
| Gripping reflex (N, pups reaching criteria) | 190 | 182 | 192 | 135 | 199 | 191 | 190 | 181* |
| Pupil constriction (N, pups reaching criteria) | 189 | 182 | 192 | 134 | 199 | 190 | 189 | 184* |

[1]p<0.05, *p<0.01

Table 53: Anophthalmia and hydrocephaly in pups in F1b.

| | | Control | 500 ppm | 1500 ppm | 5000 ppm |
|---|---|---|---|---|---|
| Anophthalmia | | | | | |
| Pup incidence | N | 0 | 0 | 1 | 0 |
| | % | 0.0 | 0.0 | 0.3 | 0.0 |
| Litter incidence | N | 0 | 0 | 1 | 0 |
| | % | 0.0 | 0.0 | 4.0 | 0.0 |
| Hydrocephaly | | | | | |
| Pup incidence | N | 0 | 0 | 1 | 0 |
| | % | 0.0 | 0.0 | 0.3 | 0.0 |
| Litter incidence | N | 0 | 0 | 1 | 0 |
| | % | 0.0 | 0.0 | 4.0 | 0.0 |

Table 54: Pup body weight changes (g) in F1a and F1b.

| Parameter | F1a | | | | F1b | | | |
|---|---|---|---|---|---|---|---|---|
| | Control 0 ppm | 500 ppm | 1500 ppm | 5000 ppm | 0 ppm | 500 ppm | 1500 ppm | 5000 ppm |
| Males | | | | | | | | |
| Days 1-4 | 2.7 | 2.8 | 2.7 | 1.3* | 2.9 | 3.1 | 2.7 | 1.6* |
| % of control | | | | 48% | | | | 55% |
| Days 4-21 | 45.5 | 44.9 | 44.2 | 27.5* | 46.3 | 46.6 | 44.9 | 29.1* |
| % of control | | | | 60% | | | | 63% |
| Females | | | | | | | | |
| Days 1-4 | 2.6 | 2.7 | 2.6 | 1.2* | 2.8 | 3.0 | 2.8 | 1.6* |
| % of control | | | | 46% | | | | 57% |
| Days 4-21 | 43.2 | 43.4 | 41.7 | 26.4* | 43.8 | 44.5 | 42.8 | 28.0* |
| % of control | | | | 61% | | | | 64% |

*p<0.01

Table 55: Selected pup physical development and reflex data (F2).

| **Parameter** | **F2** | | | |
|---|---|---|---|---|
| | **0 ppm** | **500 ppm** | **1500 ppm** | **5000 ppm** |
| Auditory canal opening (N, pups reaching criteria) | 162 | 140[1] | 170 | 105[*] |
| Eye opening (N, pups reaching criteria) | 168 | 152[1] | 159* | 116* |
| Gripping reflex (N, pups reaching criteria) | 169 | 159 | 180 | 126[1] |

[1]p<0.05, *p<0.01

**Three-generation study**

**Test guideline and GLP**

The study was performed before mandatory GLP requirements. Acclimation time and the exact age of F0 animals at the start of dosing were not mentioned, only initial age was given. It was not mentioned if sibling relationships were known. The identification system of animals was not described in detail. According to OECD 416 each test and control group should contain a sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition. For the second litter in F0, F1 and F2 generations number of pregnant animals were less than 20 in all dose groups. According to OECD 416, for the dietary studies the dose interval should not be more than 3 fold. This is not met between low and mid dose groups. The animals in F0 received test substance 8 weeks before the first breeding test. This does not meet the requirement of 10 weeks in OECD 416 (one complete spermatogenetic cycle not covered).

Not reported if the cause of infertility was determined or not. Detailed clinical examination not conducted on weekly basis. Only initial and final weights reported (weight during gestation and lactation missing). At necropsy organ weights of parental animals were not measured, only results for F3 generation presented. Sperm parameters as according to OECD 416, were not measured. Based on the information from the study report there is still uncertainty regarding the purity of the test substance. However, it was reported in the DAR that the purity is 57.9%. There was no information about analysis of test substance stability and homogeneity in the feed in the study report.

**Materials and methods**

In a three generation reproduction study (1979), mepiquat chloride (batch no.: not stated) was administered to groups of 40 male and 40 female Sprague-Dawley rats (F0) in the diet at nominal concentrations of 0 (control), 319.1, 1063.8 and 3191.5 ppm (approximately 30, 100 and 300 mg/kg/b.w./day). The test compound administration of the F0 generation animals began 8 weeks prior to mating and then constantly through all generations until the end of the test.

In F0 generation, breeding tests (one male and one female) started after pretreatment period of 8 weeks. The beginning of pregnancy was considered to be the day on which sperm was found. If finding was negative, mating was repeated up to two weeks, the partners being exchanged in succession within the according test group every seven days.

The first litter was sacrificed after 14 weeks of life. Twenty F0 female rats had spontaneous second litter and the pups were brought up and used as F1 generation (40 males and 40 females). The other 20 F0 females were laparotomised at the 20$^{th}$ day of pregnancy and examined for possible teratogenic properties. With the rats at F1 generation, breeding tests were performed likewise as well as with the F2- generation. Twenty animals/concentration/sex of the second litter of the F2-generation (F3 pups) were observed approximately 9 weeks and then sacrificed at the 10$^{th}$ week of life.

The examination of F0, F1 and F2 parental animals consisted of daily clinical inspections, daily estimation of food consumption and weekly body weight determination. The following fertility parameters were examined for the F0, F1 and F2 generation: fertility, breeding capacity, development of pups during the first 3 weeks of life, number of copulation attempts, mean duration of pregnancy, number, gender, viability, body weight and behaviour of pups determination of stillborn pups, runts, pups with malformations. The following indices were determined: fertility, gestation, viability and lactation index. The 20 dams of the F0, F1 and F2 generation which had no second litter were laparotomised at day 20 of pregnancy and subjected to various examinations/determinations such as counting of foetuses and placenta, determination of viability and gender of foetuses, number and size of resorptions, corpora lutea, implantations and location of foetuses in the uterine horns, weight of foetuses and placenta, inspection for external damage such as malformation. After dissection the foetuses were examined for possible effects on internal organs and skeleton after alizarin staining. The following parameters were determined: Corpora lutea, implantations, foetuses (absolute, per dam, distribution in the uterine horns in % in each case), dead foetuses, runts and malformed foetuses (absolute, per dam each), malformation rate, resorption rate, variation rate, pre- and post-implantation loss.

Before dissection the eyes were examined with an ophthalmoscope on parent animals only. Pups were inspected macroscopically. The auditory acuity was checked with a simple noise test. The dentition was examined. At the end of every second breeding interval, the F0-, F1- and F2- parent animals were subjected to gross examination. The offspring, which were not assigned for the next litter, were sacrificed after 3 weeks of lactation and inspected macroscopically. With F2-offspring an examination of external development (cutting of the teeth, opening of the eyes, development of the ears, beginning of the hair growth) was made.

The litter of the F3 generation, which were sacrificed after 9 weeks, were subjected to macroscopical examination and of 10 animals/group/sex (F3 generation) eleven organs (heart, liver, lungs, spleen, kidney, adrenal, thymus, pituitary, gonads, thyroid, brain) were determined. Histological examination of 30 organs of high dose and control groups of F3 generation were made. Altogether seven organs were examined also for low and mid dose groups. In parent animals only the gonads were examined in 10 animals/group/sex/generation. Where indicated statistical analysis was performed.

**Results**

Analysis of the diet conducted four times during experimental phase confirmed concentration of the active substance in the diet >90% of the nominal concentration.

A F0 male rat (control group) and a F1 male rat (mid dose group) died prematurely during weeks 33 and 34 for localized bronchopneumonia. It was reported that no changes in behavior and external appearance of the F0-, F1- and F2-generations were seen which could be attributed to exposure to the test substance. Also, it was reported that there were no intergroup variations in mean food consumption for males and females of F0-, F1-, F2- and F3- generation.

The weights of the treated rats did not differ remarkably from controls in F0-, F1-, F2- and F3- generations.

## Adverse effects on sexual function and fertility

No definite influence on either fertility or breeding results was seen at any of the tested concentrations.

## Adverse effects on development

The prenatal development of rats was not influenced in treated groups at any generation. The one observed malformation was not further described.

It was reported that no changes were observed in ophthalmological examination, simple noise test and dentition. Partially indurated lungs and single whitish foci and soft deposits/possibly sedimentation in cooled-off urine were seen in a large number of parent animals and rats of the F3-generation at all dose levels and in controls. The histological examination of gonads in 10 parent animals/group/sex of the F0-, F1- and F2-generation did not reveal any pathological changes.

**Conclusions**

Under the conditions of the study the parental and reproductive NOAELs are 3191.5 ppm (approximately 300 mg/kg bw/day), based on absence of treatment related changes. NOAEL for offsprings is 1063.8 ppm (approximately 100 mg/kg bw/day) based on reduced thymus weight, increased gonads weight and increased number of necrotic foci on livers of the females on F3 offspring at high dose group. It should be noted however, that the purity of the test substance is uncertain and it is not sure if correction for NOAELs is needed. Based on the deviations compared to OECD 416 (2001) the study is supportive at the most.

## 10.10.1 Developmental toxicity

Study 2 (rat)

**Test guideline and GLP**

The study was conducted in accordance with principles of GLP and mainly in accordance with OECD guideline 414 (2001). Administration of test substance was only on days 6-15 p.c, when according to OECD 414 administration should be from day 5 to the day prior to scheduled caesarean section. Historical control data was provided (submitted afterwards). The HC studies were dated 1 January 1991 to 31 December 1995.

**Materials and methods**

In a developmental toxicity study (1992) groups of 25 mated female Wistar rats were administered by gavage mepiquat chloride (Batch No.: WW 262/CP 1490, Purity: 57.9 % and 44.3% water) in distilled water at dose levels of 0 (vehicle control) 50, 150 and 300 mg/kg bw/day, from days 6 to 15 (inclusive) of presumed gestation (post-insemination). The purity of test substance was taken into account in dosing; concentrations used were 0.86, 2.59 and 5.18 g/100 ml and volume was 10 ml/kg. The animals were considered to be fertilized if sperm was detected in the vaginal smear and this day was designated day 0. On day 20 p.c. all surviving animals were sacrificed, fetuses were dissected from the uterus and further investigated.

Body weights were recorded regularly throughout the study period on days 0, 1, 3, 6, 8, 10, 13, 15, 17 and 20 p.c. Food consumption was determined on the same days with the exception of day 0. The animals were examined for clinical symptoms and mortality at least once a day.

After the dams had been sacrificed they were necropsied and assessed by gross pathology. Following data were recorded: weight of uterus before it was opened, number of corpora lutea, number and distribution of implantation site classified as live fetuses/dead implantations. Also conception rate, pre- and post-implantation loss were determined.

At necropsy each fetus was weighed, sexed and examined macroscopically. Also the viability of the fetuses, condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded. Soft tissue and skeletal examination of the fetuses were carried out.

It was reported that the doses were selected based on the results of a range-finding study in which dams were administered mepiquat chloride at dose levels of 100, 300 and 600 mg/kg bw/day from day 6 – 15 of presumed gestation. In the range finding study, a dose of 600 mg/kg bw/day caused marked maternal toxicity and was lethal to 50 % of the dams. The intermediate dose of 300 mg/kg b.w/day caused marked signs of toxicity including reduced body weight gain and overt signs of toxicity. However, no mortality occurred in the dams and 300 mg/kg bw/day was chosen for the high dose of the main study. No substance related effects were noted at the low dose of 100 mg/kg b.w/day.

**Results**

Concentration control analyses showed values of 96.7%-101.1% of theoretical values and in one occasion 82.5% of theoretical value. Reanalysis of the test substance after the performance of this study confirmed the stability of the active ingredient.

There were no deaths during the study. Food consumption and body weight gain were affected during the first part of treatment (day 6-13 *post coitum*) at 300 mg/kg bw/day. Also the corrected body weight gain in high dose group was reduced compared to control.

At high dose level, marked clinical signs of toxicity including tremor, unsteady gait, piloerection and hypersensitivity were noted. Symptoms were observed between days 6 to 14 of gestation. Also ataxia was observed in some animals in high dose group on days 7-9 of gestation.

The conception rate varied between 80% to 96%.

The placental and fetal weights of fetuses were comparable to control, no statistically significantly different values were observed.

At necropsy, observation only on one maternal animal on low dose group (hydronephrosis) was made.

The total fetal and litter incidence (as %) of soft tissue variations were increased at the mid and high dose groups. The fetal incidence (%) of dilated renal pelvis was increased in mid and high dose groups. Summary of fetal observations and variations is presented in the table below.

Table 56: Summary of fetal observations and variations.

| Parameter | Control | 50 mg/kg bw/day | 150 mg/kg bw/da | 300 mg/kg bw/day |
|---|---|---|---|---|
| External observations | | | | |
| Litters evaluated | 20 | 23 | 23 | 24 |
| Fetuses evaluated | 266 | 313 | 297 | 314 |
| Dead | 0 | 0 | 0 | 0 |
| Total malformations | | | | |
| Fetal incidence (N) | 0 | 0 | 1 | 1 |
| Litter incidence (N) | 0 | 0 | 1 | 1 |
| Total variations | | | | |
| Fetal incidence (N) | 0 | 0 | 0 | 0 |
| External malformations | | | | |
| Microglossia (fetal incidence, N) | 0 | 0 | 0 | 1 |
| Anophthalmia (fetal incidence, N) | 0 | 0 | 1 | 0 |
| External unclassified observations* | | | | |
| Fetal incidence (total, N) | 3 | 0 | 0 | 1 |
| Litter incidence (total, N) | 2 | 0 | 0 | 1 |
| Soft tissue observations | | | | |
| Litters evaluated | 20 | 22 | 23 | 23 |
| Fetuses evaluated | 129 | 150 | 141 | 151 |
| Dead | 0 | 0 | 0 | 0 |
| Total malformations | | | | |
| Fetal incidence (N) | 2 | 1 | 1 | 2 |
| Litter incidence (N) | 2 | 1 | 1 | 2 |
| Total variations | | | | |
| Fetal incidence (N) | 14 | 17 | 18 | 21 |
| -change to control (%) | | 121 | 129 | 150 |
| Fetal incidence (%) | 11 | 11 | 13 | 14 |
| -change to control (%) | | 0 | 118 | 127 |
| Litter incidence (N) | 10 | 11 | 13 | 14 |
| -change to control (%) | | 110 | 130 | 140 |
| Litter incidence (%) | 50 | 50 | 57 | 61 |
| -change to control (%) | - | 0 | 114 | 122 |

| | | | | |
|---|---|---|---|---|
| Soft tissue malformations and variations | | | | |
| Hydrocephaly (fetal incidence, N) | 2 | 1 | 0 | 2 |
| Malformation of great vessels (fetal incidence, N) | 0 | 0 | 1 | 0 |
| Heart, dilatation of right ventrice (fetal incidence, N) | 1 | 1 | 0 | 0 |
| Dilated renal pelvis | 14 | 17 | 18 | 21 |
| (fetal incidence, N) | - | 121 | 129 | 150 |
| - change to control (%) | | | | |
| Dilated renal pelvis | 11 | 11 | 13 | 14 |
| (fetal incidence, %) | - | 0 | 118 | 127 |
| change to control (%) | | | | |
| Hydroureter (fetal incidence, N) | 8 | 7 | 4 | 10 |
| Skeletal observations | | | | |
| Litters evaluated | 20 | 23 | 23 | 24 |
| Fetuses evaluated | 137 | 163 | 156 | 163 |
| Dead | 0 | 0 | 0 | 0 |
| Total malformations | | | | |
| Fetal incidence (N) | 3 | 7 | 11 | 7 |
| Litter incidence (N) | 3 | 6 | 6 | 6 |
| Total variations | | | | |
| Fetal incidence (N) | 68 | 69 | 57[1] | 57[1] |
| Litter incidence (N) | 20 | 22 | 22 | 20 |
| Total retardations | | | | |
| Fetal incidence (N) | 71 | 98 | 75 | 75 |
| Litter incidence (N) | 18 | 23 | 23 | 22 |
| Skeletal malformations, variations and retardations | | | | |
| Thoracic vertebral body/bodies dumbbell-shaped (asymmetr.) (fetal incidence, N) - dumbbell-shaped (asymmetr.) - bipartite (asymmetrical) | 2 | 5 | 8 | 4 |

| | | | | |
|---|---|---|---|---|
| | 0 | 2 | 0 | 1 |
| Thoracic vertebra absent (fetal incidence, N) | 1 | 0 | 0 | 0 |
| Sternebrae bipartite, ossification centers dislocated (fetal incidence, N) | 0 | 1 | 3 | 2 |
| Rib(s) absent (fetal incidence, N) | 1 | 0 | 0 | 0 |
| Sternebra(e) of irregular shape (fetal incidence, N) | 55 | 55 | 51 | 45[1] |
| Sternebra(e) bipartive (fetal incidence, N) | 5 | 3 | 0[1] | 3 |
| Accessory sternebra (fetal incidence, N) | 0 | 0 | 0 | 1 |
| Rudimentary cervical rib(s) (fetal incidence, N) | 8 | 6 | 3 | 3 |
| 13th rib(s) shortened (fetal incidence, N) | 9 | 12 | 3 | 8 |
| Accessory 14th rib(s) (fetal incidence, N) | 0 | 1 | 1 | 2 |
| Incomplete ossification (fetal incidence, N) | | | | |
| - hyoid bone | 1 | 0 | 0 | 0 |
| - skull | 1 | 0 | 2 | 0 |
| - thoracic vertebral body/bodies | 0 | 1 | 2 | 0 |
| - lumbar vertebral arch(es) | 1 | 2 | 1 | 0 |
| - sacral vertebral arch(es) | 2 | 1 | 2 | 4 |
| Thoracic vertebral body/bodies (fetal incidence, N) | | | | |
| - dumbbell-shaped (symmetr.) | 28 | 46 | 35 | 34 |
| - only one ossification center | 1 | 0 | 0 | 1 |
| Sternebrae (fetal incidence, N) | | | | |

| | | | | |
|---|---|---|---|---|
| - incompletely ossified or reduced in size | 32 | 35 | 35 | 34 |
| - not ossified | | | | |
| - only one ossification center | 13 | 15 | 5[1] | 10 |
| | 22 | 26 | 17 | 14 |

*placentae fused, blood coagulation around placenta, [1]significantly different from control, $p<0.05$

**Conclusions**

In this developmental toxicity study in rats NOAEL for maternal toxicity is 150 mg/kg bw/day, based on clinical signs of toxicity and impaired body weight gain and food consumption at 300 mg/kg bw/day. NOAEL for developmental toxicity is 50 mg/kg bw/day based on increased incidence of both total fetal and total litter soft tissue variations in mid and high dose groups. The fetal incidence (%) of dilated renal pelvis was increased in mid and high dose groups. The observed increased pre-implantation loss is considered to be not treatment related as the administration period started on day 6 of pregnancy (after implantation). Anophthalmia was observed in one pup at mid dose and hydrocephaly in one pup at low and two pups at high dose (two in control group). The study is acceptable. Historical control data report 1 case of hydrocephalus in 3901 fetuses (0.03%). Anophtalmia is not reported, but, e.g., for microphtalmia only one case is listed out of 8105 fetuses examined.

**Study 2 (rabbit)**

**Test guideline and GLP**

The study was conducted in accordance with principles of GLP. The range of temperature during housing of animals did not meet the criteria in OECD 414. According to OECD 414 each group should contain approximately 20 female animals with implantation sites at necropsy and that groups fewer than 16 animals with implantation sites may be inappropriate. In this test the number of animals was 15 in each group, which is clearly too few animals compared to guideline requirements. Administration of test substance was only on days 7-19 p.i., while according to OECD 414 administration should continue on the day prior to the scheduled caesarean section (day 28). The interval between mid and high doses does not meet the requirement of 2-4 fold interval according to OECD 414. The available historical control data is from years 1 January 1995 to 31 December 2000.

**Materials and methods**

In this developmental toxicity study (1998) groups of 15 inseminated female Himalayan rabbits were administered by gavage mepiquat chloride (Batch No.: CP028814, Purity: 56.7 %) in distilled water, at dose levels of 0, 50, 100 and 150 mg/kg bw/day, from day 7 to day 19 (inclusive) of presumed gestation (post-insemination). The control animals received doubly distilled water. The purity of test substance was taken into account in dosing; concentrations used were 1764, 3527 and 5291 mg/100 ml and volume was 5 ml/kg. The does were fertilized by means of artificial insemination and the day of insemination was designated as day 0. Observations for mortality and cageside observations for toxicity were performed daily. Food consumption was determined daily and body weights on days 0, 2, 4, 7, 9, 11, 14, 16, 19, 21, 23, 25 and 29 p.i. On day 29 post-insemination, all surviving pregnant females were killed and fetuses delivered by caesarean section. After the dams were sacrificed, they were necropsied and assessed by gross pathology. Weight of the unopened uterus, number of corpora lutea, number and distribution of implantation sites (live fetuses/dead implantations) were recorded. Conception rate and pre- and post implantation losses were calculated. At necropsy each fetus was weighed and examined macroscopically for any external findings. The viability of the fetuses, condition of the placentae, the umbilicical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded. Soft tissue and skeletal examination of the fetuses were carried out.

**Results**

Concentration control analyses showed values of 100.3%-102.0 % of theoretical values. In the 150 mg/kg bw/day dose group, two does with blood in bedding (days 23 - 27 p.i.) were observed. One of these rabbits had an early delivery (night of day 28 - 29 p.i.) and was killed on day 29 p.i. as "not scheduled sacrifice". This particular animal was found to have multiple erosions in the stomach mucosa. No weights or gestational parameters of this animal were entered into the data system. Moreover, no detailed fetal examination was possible as all fetuses of this doe were found autolytic/partly cannibalized. There were no mortalities in any of the other test groups. The mean food consumption was reduced at 150 mg/kg bw/day and 100 mg/kg bw/day dose groups on days 7-19 p.i..

At the high dose body weight loss was noted during days 7 - 19 p.i., resulting in a mean body weight loss of 50 grams over the entire treatment period. There was also a reduction in the corrected body weight gain of high dose females.

At necropsy, multiple erosions in stomach mucosa, lung edema and emphysema were noted.

In all test groups conception rate 100% was reached. The summary of reproduction data is presented in the table below. Higher percentage of live female fetuses was noted in the high dose group.

Table 57: Summary of fetal observations and variations.

| Parameter | Control | 50 mg/kg bw/day | 100 mg/kg bw/day | 150 mg/kg bw/day |
|---|---|---|---|---|
| Litters evaluated | 14 | 15 | 15 | 14 |
| Fetuses evaluated | 92 | 96 | 106 | 102 |
| Dead | 0 | 0 | 0 | 0 |
| External observations | | | | |
| Total malformations | | | | |
| Affected fetuses/litter (mean %) | 0 | 0 | 0 | 0 |
| Total variations | | | | |
| Affected fetuses/litter (mean %) | 4.1 | 1.3 | 1.7 | 0.0 |
| External variations | | | | |
| Pseudoankylosis (forelimb) Affected fetuses/litter (mean %) | 4.1 | 1.3 | 1.7 | 0 |
| Soft tissue observations | | | | |
| Total malformations | | | | |
| Affected fetuses/litter (mean%) | 3.4 | 4.3 | 2.2 | 3.4 |
| Total variations | | | | |
| Affected fetuses/litter (mean%) | 24.1 | 16.0 | 18.4 | 19.7 |

| | | | | |
|---|---|---|---|---|
| Soft tissue malformations and variations (fetal incidence (N/%)) | | | | |
| Hydrocephaly | 0/0 | 1/1.0 | 0/0 | 2/2.0 |
| Dilatation of aortic arch and aorta desc. | 0/0 | 0/0 | 1/0.9 | 0/0 |
| Septal defect | 1/1.1 | 0/0 | 2/1.9 | 0/0 |
| Abnormal position of gallbladder | 0/0 | 1/1.0 | 0/0 | 0/0 |
| Agenesia of gallbladder | 1/1.1 | 2/2.1 | 0/0 | 1/1.0 |
| Separated origin of carotids | 19/21 | 12/13 | 20/19 | 19/19 |
| Heart: traces of interventric. foramen/septum membranaceum | 4/4.3 | 1/1.0 | 2/1.9 | 3/2.9 |
| Skeletal observations | | | | |
| Total malformations | | | | |
| Affected fetuses/litter (mean%) | 4.8 | 3.1 | 1.7 | 0.8 |
| Total variations | | | | |
| Fetal incidence (N) | 16 | 19 | 23 | 25 |
| -change to control % | - | 119 | 144 | 156 |
| Fetal incidence (%) | 17 | 20 | 22 | 25 |
| -change to control % | - | 118 | 129 | 147 |
| Litter incidence (N) | 9 | 13 | 11 | 12 |
| -change to control % | - | 144 | 122 | 133 |
| Litter incidence (%) | 64 | 87 | 73 | 86 |
| -change to control % | - | 136 | 114 | 134 |
| Affected fetuses/litter (mean%) | 18.5 | 21.0 | 22.6 | 25.7 |
| -change to control % | - | 114 | 122 | 139 |
| Total retardations | | | | |
| Affected fetuses/litter (mean%) | 61.8 | 53.3 | 58.2 | 61.9 |
| Skeletal malformations, variations and retardations (fetal incidence (N/%)) | | | | |
| Thoracic vertebrae fused and/or of irregular shape | 0/0 | 0/0 | 0/0 | 1/1.0 |
| Lumbar vertebrae fused and/or of irregular shape | 2/2.2 | 0/0 | 2/1.9 | 1/1.0 |
| Lumbar vertebra absent | 0/0 | 2/2.1 | 0/0 | 0/0 |

| | | | | |
|---|---|---|---|---|
| Sternebrae severely fused (bony plate) | 1/1.1 | 0/0 | 0/0 | 0/0 |
| Splitting of skull bones | 3/3.3 | 2/2.1 | 7/6.6 | 6/5.9 |
| Epactal bone between nasal and frontal bones | 2/2.2 | 6/6.3 | 4/3.8 | 7/6.9 |
| Epactal bone between parietal bones | 0/0 | 0/0 | 1/0.9 | 1/1.0 |
| Accessory thoracic vertebra | 0/0 | 0/0 | 1/0.9 | 2/2.0 |
| Sternebrae fused | 5/5.4 | 3/3.1 | 4/3.8 | 5/4.9 |
| Sternebrae of irregular shape | 2/2.2 | 3/3.1 | 5/4.7 | 2/2.0 |
| Accessory sternebra | 0/0 | 2/2.1 | 0/0 | 0/0 |
| Accessory 13th rib(s) | 5/5.4 | 2/2.1 | 6/5.7 | 9/8.8 |
| 12th rib(s) shortened | 1/1.1 | 2/2.1 | 0/0 | 1/1.0 |
| Rudimentary cervical rib(s) | 0/0 | 1/1.0 | 1/0.9 | 0/0 |
| Incompletely ossified | | | | |
| -hyoid bone | 31/34 | 17/18 | 19/18 | 35/34 |
| -Interparietal and/or parietal | 3/3.3 | 0/0 | 3/2.8 | 2/2.0 |
| bones | 7/7.6 | 1/1.0 | 7/6.6 | 7/6.9 |
| -cervical vertebral body/bodies | 2/2.2 | 0/0 | 2/1.9 | 0/0 |
| -thoracic vertebral body/bodies | 1/1.1 | 2/2.1 | 3/2.8 | 0/0 |
| -lumbar vertebral arch(es) | 1/1.1 | 0/0 | 1/0.9 | 0/0 |
| -sacral vertebral body/bodies | 1/1.1 | 1/1.0 | 4/3.8 | 0/0 |
| -sacral vertebral arch(es) | 1/1.1 | 1/1.0 | 1/0.9 | 1/1.0 |
| -rib(s) | 1/1.1 | 0/0 | 1/0.9 | 1/1.0 |
| -talus | 1/1.1 | 0/0 | 0/0 | 0/0 |
| -os pubis | | | | |
| Sternebrae incompletely ossified or reduced in size | 19/21 | 21/22 | 17/16 | 32/31 |
| Not ossified | | | | |
| -cervical vertebral body/bodies | 1/1.1 | 0/0 | 0/0 | 0/0 |
| -sternebrae | 16/17 | 18/19 | 23/22 | 14/14 |
| -talus | 1/1.1 | 0/0 | 0/0 | 0/0 |

| | | | | |
|---|---|---|---|---|
| Thoracic vertebr. body/bodies, only one ossification center | 0/0 | 0/0 | 0/0 | 1/1.0 |

NOAEL for developmental toxicity is <50 mg/kg bw/day, based on increased fetal and litter incidence of total skeletal variations. Hydrocephaly was observed in low (1 fetus) and high dose (2 fetuses) groups. The historical control data reported one case of hydrocephalus out of 2459 fetuses examined (0.08%).

**Study 3 (rat), not reliable**

**Test guideline and GLP**

The study preceded mandatory GLP requirements. Details of animal identification system were not given. Test substance purity was not provided. Gravid uteri weight was not measured. In addition to administration on days 0-20 p.c., a group of 10 animals were administered on days 0 p.c.- 21 post partum. This part of the study does not follow OECD 414 or 416 and animal number is lower than recommended.

**Materials and methods**

In this developmental toxicity study (1977) groups of 25 mated female Sprague-Dawley rats were administered 1,1-dimethylpiperidinium chloride (mepiquat chloride, compound No.: XXVI/408, Purity: not stated) at dose levels of 0, 100, 300, 1000 or 3000 ppm in the diet days 0 – 20 after insemination. These rats were sacrificed on the 20$^{th}$ day p.c. and the fetuses were delivered by C-section.

In a further investigation groups of 10 mated animals received in the diet the same dose from day 0 after insemination until day 21 post partum. These animals were were allowed to give birth spontaneously and rear the pups up to the 21$^{st}$ day post partum, then all the dams and pups were sacrificed. The day when sperm was detected in the vaginal smear was considered day 0 of pregnancy.

For the study segment with administration from day 0 to 20$^{th}$ day post coitum, food consumption was determined each day and body weight three times a week. The animals were checked each day for clinical symptoms and mortality. After sacrifice dams were examined for any macroscopically recognizable pathological changes. Conception rate was calculated, number of corpora lutea determined and site, total number and the mean number of implants were determined. Viable and dead implants were recorded as well as early, intermediate and late resorptions, dead foetuses and percentage of dead implants. Foetuses were examined macroscopically, their weight and length were recorded as well as the placenta weight. From 2/3 of viable fetuses for each dam skeletons were examined for variations, retardations and malformations. From the remaining 1/3 of the fetuses deformities, variations and retardations of the organs were examined.

For the study segment with administration from day 0 to 21st day post partum, food consumption of dams was determined each day and body weights on day 0, three times a weekly during the prenatal period and once a week during the post natal period. The animals were checked each day for clinical symptoms and mortality. The delivery behavior was observed and the number and sex of the fetuses delivered was established. After sacrifice the dams were examined for any macroscopically recognizable pathological changes. Conception rate was calculated, implantations determined and the uteri of all animals were checked for resorption sites. The weights of heart, liver, kidneys and spleen of the dams were determined. The body weight of the pups was determined on their birth and on days 7, 14 and 21 post partum. The pups were examined for beginning of hair growth, opening of eyes, behavior in the litter and clinically recognizable symptoms of poisoning. Mortality was checked each day. The assessment of skeletons of dead pups covered deformities, variations and retardations. The pups which died up to the 14$^{th}$ day post natum and the pups which died after the 14$^{th}$ day post natum were examined by different methods. Also the organs of dead pups were examined. Viability index of the pups was calculated. Various behavioural studies, a swimming test and rotational bar test were carried out. After sacrifice animals were examined macroscopically, the weights of heart, liver, kidneys and spleen of the pups were determined. The heads and skeletons of the sacrificed pups were assessed. The assessment of skeletons was based on the X-ray pictures and covered deformities, variations and retardations.

**Results**

The study segments with administration from day 0 to 20th day post coitum

Food consumption and body weight development were comparable between treated and control groups at all dose levels tested. It was reported that no symptoms of poisoning were found clinically in any of the animals. There were no mortalities.

It was reported that macroscopical assessment revealed a considerably enlarged right kidney with severe cystic dilation of the renal pelvis on one animal at dose group 100 ppm.

Conception rate was slightly decreased in 3000 ppm group compared to control. Early resorptions were detected in all test groups, with increased incidence in 100, 300 and 3000 ppm groups. Intermediate resorptions were detected in all treated groups but not in the control group.

The length and weight of foetuses were comparable between treated and control groups. The mean weight of placentae of females in the 3000 ppm group was decreased compared to control.

The malformations, variations and retardations observed in different groups are presented in the table below.

Table 58. Malformations, variations and retardations in groups with administration period of days 0-20 p.c.

| Parameter | Control | 100 ppm | 300 ppm | 1000 ppm | 3000 ppm |
|---|---|---|---|---|---|
| Viable fetuses | 175 | 207 | 202 | 190 | 200 |
| Examinations (Dawson) | 116 | 138 | 135 | 126 | 134 |
| Examinations (Wilson) | 59 | 69 | 67 | 64 | 66 |
| No. of malformations | | | | | |
| Foetuses | 12 | 4 | 4 | 4 | 2 |
| % viable fetuses/litter | 11.29 | 1.67* | 1.55 | 3.03 | 1.41* |
| Litters | 9 | 2 | 4 | 3 | 2 |
| Litters % | 42.86 | 10.0 | 19.05 | 14.29 | 10.53* |
| Observed malformations | | | | | |
| Vertebral column,<br>Bipartite thoracic vertebra centra<br>Bipartite and assymetrical thoracic vertebra centra | 3 | 1<br>1 | 3 | 1 | 1 |
| Ribs, Wavy ribs bilateral<br>Wavy ribs unilateral | 4<br>4 | 2<br>1 | <br>1 | 2<br>1 | 1 |
| Thorax, hernia of the diaphragm | 1 | | | | |
| Liver, enlarged liver | 1 | | | | |
| No. of variations and retardations | | | | | |
| Foetuses | 21 | 27 | 21 | 22 | 21 |
| % viable fetuses/litter | 13.47 | 11.36 | 10.04 | 10.82 | 10.22 |
| Litters | 10 | 13 | 13 | 12 | 12 |

| | | | | | |
|---|---|---|---|---|---|
| Litters % | 47.62 | 65.0<br>136% of control | 61.90<br>130% of control | 57.14<br>120% of control | 63.16<br>133% of control |
| Observed variations and retardations | | | | | |
| Skull, incomplete ossification of skull bones | 9 | 4 | 7 | 8 | 4 |
| Ribs, accessory ribs bilateral | 1 | 2 | 2 | 3 | 1 |
| Sternum, absent sternebrae<br>partially ossified sternebrae<br>asymmetrical sternebrae | 1<br>9<br>1 | 3<br>16<br>4 | 3<br>9<br>2 | 2<br>8 | <br>12<br>2 |
| Certebral column, partially ossified thoracic vertebra centra | | | | | 1 |
| Kidney, enlarged renal pelvis bilateral<br>Enlarged renal pelvis unilateral | | | | <br>3 | 3<br>1 |
| Generalized retardation | | | 1 | 1 | 1 |

*Significance 95%

<u>The study segment with administration from day 0 to 21st day post partum</u>

There was a decrease in food consumption of dams in all dose levels during first days of the study, post coitum.

Body weight change was decreased compared to controls during days 0-15 post coitum.

It was reported that no compound-induced symptoms of poisoning were detectable clinically in any of the test animals. There were no mortalities. It was reported that at autopsy in one animal of the 100 ppm group amber coloured liquid was found in thorax, haemorrhaegic ulcerations was observed in caecum, the edges of the liver were slightly blunted and the left kidney was observed to have a yellowish white area which tapered into the medullary zone. It was reported that there was no change seen in the delivery behavior of the animals, no difference could be determined in the delivery date of the animals in any of the test groups.

The body weight of female pups was slightly decreased compared to control in all dose groups during days 0-21.

It was reported that no symptoms of poisoning to the animals could be detected clinically, their coats began to grow on the 7th day post natum and the eyes of the animals in all the test groups opened between the 14th and 16th day post natum.

The survival index of pups was decreased compared to control at 1000 ppm and 3000 ppm groups towards the end of the study. In the assessment of skeletons and organs of dead pups it was reported that e.g. accessory piece of bone fused with a sternal bone and accessory sternebrae at 1000 ppm group and accessory rib and fusion of individual sternal bones were observed at 3000 ppm group.

It was reported that at autopsy cystic dilation of the renal pelvis was detected in one pup at 3000 ppm group. The heart weight of males and absolute spleen, liver and kidney weights of females were decreased at the 3000 ppm group.

An increased incidence of fused accessory sternebrae compared to control was observed in all test groups. Various pups in all test groups were found to have a cyst in the brain.

**Conclusions**

The purity of the test substance was not provided.

In the first part of the study, administration period of days 0-20 p.c., the number of fetuses with malformations was increased in control group compared to treated groups. 42.86 % of control litters contained malformations. This number remarkably exceeds the number observed in treated groups. In addition, a high number of affected litters (47.62%) containing variations and retardations were observed in control animals. Therefore the study is considered not acceptable.

The latter part of the study does not follow OECD 414 or 416 and has fewer animals than recommended.

## 10.10.2Adverse effects on sexual function and fertility

### Table 59: 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 |
|---|---|---|---|
| OECD 416 (2001).<br>- GLP<br>- 58% mepiquat/ 44% water<br>- Wistar rats (25m+25 f)<br>-.<br>- At necropsy, the weight of uterus, ovaries, prostate, seminal vesicles, brain, spleen, pituitary, thyroid and adrenal glands were not measured on parental animals.<br>- Deviations in measurements of sperm counts, oestrus cycle and sexual maturation<br>- It was not reported if detailed testicular histopathology and qualitative/quantitative evaluation of primordial follicles were conducted.<br>- Organ weights of pups not determined at necropsy. | 0, 500, 1500, 5000 ppm<br>active ingredient; 0, 864, 2591 and 8636 ppm test substance<br>F0: Treatment at least 70 before mating<br>F1: Treatment at least 98 days before mating | Parental:<br>Reduced body weight in females during lactation of F2 pups<br>Adverse effects on sexual function and fertility<br>Effects on gestation time<br><br>Offspring (adverse effects on development):<br>Depressed survival rate, lower viability and lactation indices, effects on body weight gain and morphological development (pups reaching the criteria of auditory canal opening, eye opening and gripping reflex), anophthalmia and hydrocephaly observed in one pup at mid dose.<br>Total number of litter, total number of pups delivered and liveborn pups.<br>The fertility indices for F0 males/females were: 100 (0 ppm), 92 (500 ppm), 100 (1500 ppm), 80 (5000 ppm) for F1a litters and 100 (0 ppm), 96 (500 ppm), 100 (1500 ppm), 100 (5000 ppm) for F1b.<br>The respective gestation times were, 22d (0 ppm), 22d (500 ppm), 22d (1500 ppm) and 21.6d** for the 5000 ppm F1a pups.<br>The gestation times were 21.9d (0 ppm), 21.9d (500 ppm), 21.8d (1500 ppm) and 21.6d* for the 5000 ppm F1a pups.<br>*p<0.05, **p<0.01<br>The fertility indices for the F1 males were: 92 (0 ppm), 88 (500 ppm), 92 (1500 ppm) and 83 (5000 ppm) for the F2 litters.<br>The F0 relative testes weight were: 0.724 (0 ppm), 0.71 (500 ppm), 0,727 (1500 ppm) 0.819 (5000 ppm)* The high dose weight was 113% of control.<br>The relative epididymis weights for the F0 were: 0.25 (0 ppm), 0.25 (500 ppm), 0.26 (1500 ppm) and 0.28 (5000 ppm). The high dose | 1993 |

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| | | weight was 112% of control.<br>(*p<0.01)<br>The F1 relative testes weight were: 0.765 (0 ppm), 0.744 (500 ppm), 0.765 (1500 ppm), 0.854* 112% of control (5000 ppm)<br>The relative epididymis weights for the F1 were: 0.26 (0 ppm), 0.256 (500 ppm), 0.255 (1500 ppm), 0.303 (5000 ppm)* (116% of control) (*p<0.01) | |
| - 3-generation<br>- Pre-GLP<br>- Sprague Dawley rat<br>-<br>-<br>- F1 and F2 generations number of pregnant animals were less than 20 in all dose groups.<br>-The pre-breeding treatment of F0 8 weeks instead of 10 weeks in OECD 416 (one complete spermatogenetic cycle not covered).<br>-<br>- Detailed clinical examination not conducted on weekly basis. Only initial and final weights reported (weight during gestation and lactation missing).<br>- At necropsy organ weights of parental animals were not measured, only results for F3 generation presented.<br>- Sperm parameters were not measured according to OECD 416 Based on the | 0, 319.1, 1063.8, 3191.5 ppm, F0 treatment 8 weeks prior to mating and then constantly through all generations until the end of the test. | Adverse effects on sexual function and fertility<br>Absence of treatment related changes.<br>Adverse effects on development:<br>Changes in organ weights, histopathological findings in liver (necrotic foci). The F3 high dose males had 112% increased gonad weight. | 1979 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| information from the study report there is still uncertainty regarding the purity of the test substance. However, it was reported in the DAR that the purity is 57.9%. | | | |

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

In the 2-generation study there was shorter gestation time for the F1a and F1b litter for high dose dams. This effect was not seen in the F2 litter. The total number of litter, total number of pups delivered and liveborn pups were reduced compared to control on F1 and F2 generation. Fertility indices for F1 males and females were slightly decreased in the high dose group compared to controls.

In the 2-generation study some effects on development were observed. Those effects are discussed under developmental toxicity.

### 10.10.4 Comparison with the CLP criteria

According to the CLP Regulation (1272/2008):

*3.7.1.3. Adverse effects on sexual function and fertility*

*Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.*

CATEGORY 1

*Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).*

Category 1A

*"Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans."*

The substance does not meet the criteria for classification for fertility cat. 1A because the effects or their severity observed are not sufficient for classification.

Category 1B

*"Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.*"

The substance does not meet the criteria for classification for fertility cat. 1B because the effects or their severity observed are not sufficient for classification.

*Category 2*

"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and 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. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*"

The substance does not meet the criteria for classification for fertility cat. 2 because the effects or their severity observed are not sufficient for classification.

### 10.10.5 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** | **Results** | **Reference** |
|---|---|---|---|
| OECD 416 | 0, 500, 1500, 5000 ppm | Depressed survival rate, lower viability and lactation indices, effects on body weight gain and morphological development (pups reaching the criteria of auditory canal opening, eye opening and gripping reflex), anophthalmia and hydrocephaly observed in one pup at mid dose.<br>Total number of litter, total number of pups delivered and liveborn pups. | 2001 |
| 3-generation<br>Pre-GLP<br>Sprague Dawley rat | 0, 319.1, 1063.8, 3191.5 ppm, F0 treatment 8 weeks prior to mating and then constantly through all generations until the end of the test. | Changes in organ weights, histopathological findings in liver | 1979 |
| Developmental toxicity study<br>OECD 414 (2001)<br>GLP | 0, 50, 150, 300<br>mg/kg bw/day | Maternal (300 mg/kg bw/day):<br>Impaired food consumption and reduced body weight gain clinical signs of toxicity including tremor, unsteady gait, piloerection and hypersensitivity during days 6 to 14 of gestation | 1992, 1997 |

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, dose levels duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| Wistar rats, n=25 | | Developmental:<br>Increased incidence of both total fetal and total litter soft tissue variations. Anophthalmia was observed in one pup at mid dose and hydrocephaly in one pup at low and two pups at high dose. | |
| Developmental toxicity study<br>OECD 414<br>GLP<br>Himalayan rabbits<br>- Animal number 15 instead of 20 | 0, 50, 100, 150 mg/kg bw/day<br>- exposure only during days 7-19 post implantation | Maternal:<br>Reduced food consumption and body weight gain at mid and high doses.<br>Developmental:<br>Increased fetal and litter incidence of total skeletal variations<br>Hydrocephaly was observed in low (1 fetus) and high dose (2 fetuses) groups. | 1998 |
| Developmental toxicity (pre-, peri- postnatal toxicity) study Sprague-Dawley rat<br>- Pre-GLP<br>- Pre-OECD guideline<br>- . Details of animal identification system were not given. Test substance purity was not provided. Gravid uteri weight was not measured. | 0, 100, 300, 1000, 3000 ppm<br>- 25/group animals days 0 – 20 after insemination (C-section)<br>- 10 animals/group, day 0 after insemination until day 21 post partum (Natural birth) | The malformation rate of the control group was over 4x higher than in the high dose group. The reliability of this study is questionable. | 1977 |

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

In the 2-generation study some effects on development were observed. Mean body weight gains of high dose F1a, F1b and F2 pups were reduced and the number of F1a, F1b and F2 pups reaching the criteria of auditory canal opening and eye opening and gripping reflex of F1b pups was decreased in the high dose group. A high number of pups in F1a high dose group died. In addition to depressed survival rate, the F1 pups had lower viability and lactation indices, effects on body weight gain and morphological development (pups reaching the criteria of auditory canal opening, eye opening and gripping reflex), anophthalmia and hydrocephaly observed in one pup at mid dose.

In the developmental toxicity study on rat increased incidence of both total fetal and total litter soft tissue variations in mid and high dose groups were observed. Anophthalmia was observed in one pup at mid dose and hydrocephaly in one pup at low and two pups at high dose (incidence for hydrocephaly comparable to control).

In the developmental study on rabbit an increased fetal and litter incidence of total skeletal variations and hydrocephaly (1 animal low dose, 2 animals high dose) were observed. In the 2-generation study on rat anophthalmia and hydrocephaly were observed both in the same F1b pup of mid dose group (1500 ppm). Taken together, these effect give an indication of an effect to development of pups and would support classification as **Repr. 2; H361d** (Suspected of damaging the unborn child).

### 10.10.7 Comparison with the CLP criteria

According to the CLP Regulation (1272/2008):

*3.7.1.4. Adverse effects on development of the offspring*

*Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.*

CATEGORY 1

*Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).*

Category 1A

*"Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans."*

The substance does not meet the criteria for classification for development cat. 1A because the effects or their severity observed are not sufficient for classification.

Category 1B

*"Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.*"

The substance does not meet the criteria for classification for development cat. 1B because the effects or their severity observed are not sufficient for classification.

*Category 2*

"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and 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. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.*"

There is evidence from reliable developmental toxicity studies two species that mepiquat causes fetal malformations, namely, hydrocephalus and anophtalmia. These findings are supported by similar developmental malformations found in pups of the 2-generation reproductive toxicity study. Even though these malformations are somewhat rare, they do not show a clear dose response pattern. Some maternal toxicity was present mostly demonstrated by decreased body weight gain. However, it is unlikely that this had a significant impact on these types of malformations. Therefore, classification **Repr. 2; H361d** (Suspected of damaging the unborn child) is proposed.

### 10.10.8 Adverse effects on or via lactation

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

The data does not allow the evaluation of effects via lactation.

### 10.10.10 Comparison with the CLP criteria

### 10.10.11 Conclusion on classification and labelling for reproductive toxicity

Classification Repr. 2; H361d (Suspected of damaging the unborn child) is proposed. No classification is proposed for fertility effects. Specific target organ toxicity-single exposure

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

Reproductive toxicity of mepiquat chloride was investigated in several guideline studies: a 2-generation study in rats, a rat PNDT study, a rabbit PNDT study, and a developmental neurotoxicity study in rats. Two pre-guideline studies are also available.

***Sexual function and fertility***

Since the severity of effects on sexual function and fertility in the 2-generation study was not considered sufficient by the DS, they proposed no classification for this endpoint.

***Development***

The DS proposed classification in Category 2 mainly based on hydrocephalus and anophthalmia in the rat and rabbit studies:

- Rabbit PNDT study (1998): foetal (litter) incidence of hydrocephaly was 0, 1, 0, 2(1) in the control, low-, mid-, and high-dose group respectively
- Rat PNDT study (1992): foetal (litter) incidence of hydrocephaly 2(2), 1, 0, 2(2); foetal incidence of anophthalmia 0, 0, 1, 0
- Rat 2-generation study (1993): hydrocephaly and anophthalmia in a single pup at the mid-dose

The DS further mentioned reduced pup body weight, increased pup mortality and effects on morphological development in the 2-generation study, an increase in total soft tissue variations in the rat PNDT study, and an increase in total skeletal variations in the rabbit PNDT study.

***Lactation***

According to the DS the data does not allow evaluation of effects via lactation.

### Comments received during consultation

Comments were received from 3 MSCAs and a manufacturer. None of the commenters addressed sexual function and fertility. As to development, 2 MSCAs supported Category 2 whereas 1 MSCA and a manufacturer preferred no classification. The MSCA proposing no classification pointed out the low incidence of malformations and lack of a dose-response relationship. The manufacturer's argumentation can be summarized as follows:

- Rat 2-generation study: hydrocephaly and anophthalmia in a single mid-dose F1b pup is most likely a spontaneous finding, as indicated the by the HCD range and by absence of similar findings in other dose groups (especially the top dose) and other cohorts (F1a, F2). Reduced pup body weight, delayed development and increased pup mortality (F1a only) during lactation are secondary non-specific consequences of marked maternal toxicity at the top dose (reduced maternal food consumption and body weight gain, clinical signs of toxicity).

- Rat PNDT study: foetal and litter incidence (as %) of hydrocephaly is higher in the concurrent control than at the top dose. The single incidence of anophthalmia at the mid-dose is a spontaneous finding. The increases in total soft tissue variations and dilated renal pelvis were not statistically significant and the values were well within the HCD ranges.
- Rabbit PNDT study: the litter incidence of hydrocephaly does not show a dose-response relationship and is within the HCD range. The increase in total skeletal variations was not statistically significant and the values were well within the HCD range.

## Assessment and comparison with the classification criteria

***Adverse effects on fertility and sexual function***

Two-generation study in rats (1993)

This study was conducted under GLP and in line with OECD TG 416 (1983). Several parameters added into OECD TG 416 in 2001, such as sperm parameters or puberty onset, were not investigated in this study.

Wistar rats were administered mepiquat chloride at dietary concentrations of 0, 500, 1500 and 5000 ppm (equivalent to ca. 0, 52, 155 and 550 mg/kg bw/d). The parental generation was mated two times, producing F1a and F1b litters; the F1a litter was used to produce F2 generation.

The top dose caused general toxicity manifest as parental body weight reduction by ca. 10-20% compared to control (at mating and during lactation: F0 by ca. 10-15%, F1 by ca. 20%), clinical signs of neurotoxicity during lactation (tremor and hypersensitivity in more than half of the dams, incidence peaking between postpartum days 14 and 23) and liver pathology (reduced lipid storage in the liver attributed to a catabolic nutritional situation). No general toxicity was observed at the mid-dose.

No clear effect on fertility or sexual function was identified at the top dose. A very slight reduction in gestation length in F0/F1 (by 0.3-0.4 days) was not reproduced in F1/F2. A marginal reduction in absolute testicular weight in F1 males (by 11%) was not accompanied by histopathological findings and can be at least partly attributed to a marked body weight reduction (by 20%). Histopathological examination of reproductive organs did not reveal any treatment-related changes.

A slight reduction in litter size was observed in F1/F2 at the top dose (10.7 vs 12.8 in the control; HCD range 11.1-16.4). The number of implantation sites was not determined in this study. A reduction in litter size reportedly occurred also in a one-generation range-finding study at 6000 ppm. Thus, the finding in the main study might be related to treatment. However, given the magnitude of the effect, the lack of statistical significance and presence of some maternal toxicity (maternal body weight reduction by 16% on GD 0 compared to control), this finding is not sufficient for classification. Effects on pup body weight and survival are discussed under development.

Three-generation study in rats (1979)

In this pre-guideline and pre-GLP study, Sprague-Dawley rats were administered mepiquat chloride at dietary concentrations up to 3190 ppm (equivalent to ca. 340 mg/kg bw/d). No general or reproductive toxicity was observed in this study.

Repeated dose studies

No effects on reproductive organs were observed in the available repeated dose studies in rats, mice and dogs.

Conclusion on classification for fertility and sexual function

In the absence of significant or consistent effects on sexual function and fertility in the available studies, RAC agrees with the DS's proposal of no classification.

***Adverse effects on development***

PNDT study in rats (1992)

The study was conducted under GLP and according to OECD TG 414 (1981). Mepiquat chloride was administered to pregnant Wistar rats via gavage on GD 6-15 at dose levels of 0, 50, 150 and 300 mg/kg bw/d. Dose selection was based on a preliminary study where half of the dams died after 1-2 doses of 600 mg/kg bw. The top dose of 300 mg/kg bw/d in the main study caused transient clinical signs of toxicity (tremor, piloerection, unsteady gait, hypersensitivity) mainly in the first half of the treatment period. No evidence of developmental toxicity was observed in this study according to the study authors.

The DS mentioned hydrocephaly and anophthalmia in this study as part of justification for Cat. 2. The foetal (litter) incidence of hydrocephaly was 2(2), 1, 0, 2(2) in the control, low-, mid- and high-dose respectively. Thus, there is in fact no increase in hydrocephaly in this study.

A single case of unilateral anophthalmia occurred at the mid-dose. No anophthalmia was observed in the concurrent control nor in historical controls for the rat PNDT study (25 studies within 5 years after the current study, i.e. 1991-1995) but several cases were present in the HCD for the 2-generation study (1987-1992). The PNDT study and the 2-generation study were conducted in 1991 in the same laboratory with animals of the same strain and source. The single occurrence without a dose-response relationship is likely to be a spontaneous finding and is not considered to support classification.

The DS further mentioned an increase in total soft tissue variations and specifically dilated renal pelvis. The foetal (litter) % incidence of dilated renal pelvis (as a variation) was 11(50), 11(50), 13(57) and 14(61) in the control, low-, mid- and high-dose respectively. The incidence in the treated groups was not significantly different from the concurrent control and the values were close to the HCD mean (foetuses 15%, litters 51%).

PNDT study in rabbits (1998)

The study was conducted under GLP and according to OECD TG 414 (1981). Mepiquat chloride was administered to pregnant Himalayan rabbits via gavage on GD 7-19 at dose levels of 0, 50, 100 and 150 mg/kg bw/d.

The top dose induced a decrease in food consumption (GD 7-19 by 42%) and in body weight gain. One top dose dam delivered shortly before the scheduled sacrifice (during the night from GD 28 and 29, scheduled sacrifice GD 29); this dam had multiple erosions in the stomach mucosa and showed a markedly reduced food intake prior to delivery.

Hydrocephaly was observed in 0, 1, 0 and 2(1) control, low-, mid- and high-dose foetuses (litters) respectively. A single case of hydrocephaly was reported in historical controls (10 studies within 3 years of the current study).

The DS further mentioned an increase in total skeletal variations. The litter-based incidence (% of affected foetuses per litter) of total skeletal variations was 19, 21, 23 and 26%. There was no statistically significant difference from concurrent control and the values were below the HCD mean (49%).

Two-generation study in rats (1993)

The most remarkable finding potentially related to development in this study is a dramatic effect on pup body weight gain, see the table below.

**Pup viability and body weight development in the 2-generation study**

| **Dose (ppm)** | **0** | **500** | **1500** | **5000** |
|---|---|---|---|---|
| Dose (mg/kg bw/d) – grand mean | 0 | 52 | 155 | 550 |
| Dose (mg/kg bw/d) – lactating dams | 0 | 70-79 | 200-230 | 630-720 |
| Dose (mg/kg bw/d) – F1 pups at the beginning of pre-mating period (around PND 30-40) | 0 | 93 | 280 | 1000 |
| **F0/F1a** | | | | |
| No. of litters | 25 | 23 | 25 | 20 |
| Litter size at delivery | 14.0 | 13.9 | 14.8 | 13.3 |
| No. of liveborn pups | 345 | 314 | 365 | 264 |
| Pups dead LD 0-4[a]; (litters affected) | 15 (11) | 16 (8) | 22 (7) | 30 (11) |
| No. of pups post-culling | 200 | 184 | 192 | 149 |
| Pups dead LD 5-21[a]; (litters affected) | 10 (3) | 1 (1) | 0 (0) | 13 (4) |
| Maternal bw LD 0 (g) | 305 | 304 | 310 | 272** (-11%) |
| Maternal bw LD 21 (g) | 326 | 329 | 329 | 272** (-17%) |
| Incidence of tremor in lactating dams | 0 | 0 | 0 | 17 |
| Pup bw LD 1 (g) | 6.4 | 6.5 | 6.3 | 6.0* (-6%) |
| Pup bw LD 4 pre-culling (g) | 9.1 | 9.2 | 9.0 | 7.3** (-20%) |
| Pub bw LD 7 (g) | 15.1 | 15.0 | 15.0 | 10.9** (-28%) |
| Pup bw LD 14 (g) | 32.7 | 32.7 | 33.0 | 23.8** (-27%) |
| Pup bw LD 21 (g) | 53.5 | 53.4 | 52.0 | 34.3** (-36%) |
| F1, bw at the beginning of the pre-mating period (around PND 30), males (g) | 108.5 | 104.5 | 101.9 | 55.7** (-49%) |
| F1, bw at the beginning of the pre-mating period (around PND 30), females (g) | 97.0 | 95.6 | 93.3 | 50.6** (-48%) |
| **F0/F1b** | | | | |

| | | | | |
|---|---|---|---|---|
| No. of litters | 25 | 24 | 25 | 25 |
| Litter size at delivery | 15.4 | 14.2 | 14.6 | 13.9 |
| No. of liveborn pups | 375 | 332 | 359 | 340 |
| Pups dead LD 0-4[a]; (litters affected) | 20 (12) | 8 (6) | 21 (11) | 14 (7) |
| No. of pups post-culling | 200 | 192 | 192 | 200 |
| Pups dead LD 5-21[a]; (litters affected) | 1 (1) | 1 (1) | 2 (2) | 7 (4) |
| Maternal bw LD 0 (g) | 348 | 347 | 353 | 308** (-11%) |
| Maternal bw LD 21 (g) | 358 | 360 | 363 | 306** (-15%) |
| Incidence of tremor in lactating dams | 0 | 0 | 0 | 20 |
| Pup bw LD 1 (g) | 6.4 | 6.6 | 6.5 | 6.1* (-5%) |
| Pup bw LD 4 pre-culling (g) | 9.2 | 9.7 | 9.1 | 7.7** (-16%) |
| Pub bw LD 7 (g) | 15.3 | 15.8 | 15.3 | 11.6** (-24%) |
| Pup bw LD 14 (g) | 33.5 | 34.2 | 33.1 | 23.9** (-29%) |
| Pup bw LD 21 (g) | 54.3 | 55.3 | 53.1 | 36.2** (-33%) |
| **F1/F2** | | | | |
| No. of litters | 23 | 22 | 23 | 20 |
| Litter size at delivery | 12.8 | 12.5 | 13.5 | 10.7 |
| No. of liveborn pups | 286 | 261 | 299 | 212 |
| Pups dead LD 0-4[a]; (litters affected) | 12 (8) | 18 (7) | 13 (7) | 15 (6) |
| No. of pups post-culling | 182 | 163 | 180 | 144 |
| Pups dead LD 5-21[a]; (litters affected) | 13 (5) | 4 (3) | 0 (0) | 14 (6) |
| Maternal bw LD 0 (g) | 318 | 319 | 323 | 257** (-19%) |
| Maternal bw LD 21 (g) | 326 | 326 | 325 | 254** (-22%) |
| Incidence of tremor in lactating dams | 0 | 0 | 0 | 14 |
| Pup bw LD 1 (g) | 6.3 | 6.4 | 6.3 | 6.2 |
| Pup bw LD 4 pre-culling (g) | 8.7 | 8.8 | 8.9 | 7.8 |
| Pub bw LD 7 (g) | 14.2 | 13.7 | 14.2 | 11.3** (-20%) |
| Pup bw LD 14 (g) | 31.2 | 30.3 | 30.9 | 23.9** (-23%) |
| Pup bw LD 21 (g) | 50.8 | 49.2 | 49.6 | 34.7** (-32%) |

Statistically significant difference from control: *, $p < 0.05$; **, $p < 0.01$

[a] Statistical significance not shown

The overall developmental retardation in the top dose pups is further documented by a delay in attaining of physical developmental landmarks (pinna unfolding, auditory canal opening, eye opening – normally attained by PND 4, 13 and 15 respectively). In the end the top dose animals did reach adulthood and showed a normal reproductive performance. The body weight impairment partly persisted (F1 animals had lower body weight by ca. 20% compared to controls at mating) but this was under a continued dietary exposure.

Marked developmental retardation occurred already within the first week. Such an effect can in principle result from (1) *in utero* exposure, either as a specific effect or a non-specific secondary consequence of maternal toxicity; (2) early postnatal exposure via milk; (3) a specific effect of the test substance on milk production; or (4) impaired milk production or impaired maternal care as a secondary, non-specific consequence of maternal toxicity.

The effect on postnatal development occurred only at the top dose of 5000 ppm causing overt maternal toxicity but not at the next lower, maternally non-toxic dose of 1500 ppm. Similarly, a limited 1-generation range-finding study showed pup toxicity only at maternally toxic doses of 6000 and 4000 ppm, but not at 2000 ppm where no maternal toxicity was seen. A summary of the 1-generation study can be found under 'supplemental information'.

The correlation between maternal and pup toxicity in the 2-generation study and in the 1-generation range-finding study strongly suggests that maternal toxicity played a key role, although the available information does not allow exclusion of the remaining possible causes.

The DS further mentioned reduced survival of top dose F1a pups. Although an effect on survival would be plausible given the concomitant developmental retardation and maternal toxicity, no effect on pup survival was observed in F1b or F2. Thus, it remains unclear whether the reduced postnatal viability in F1a is related to treatment.

Besides these findings, the DS pointed out occurrence of anophthalmia and hydrocephaly in this study in support of Category 2. There was a single pup with hydrocephaly and unilateral anophthalmia in a mid-dose F1b litter (sacrifice on PND 21). Other pups in this litter appeared normal. No other cases of hydrocephaly or anophthalmia were reported in this study. 5 cases of anophthalmia (3 litters) and 1 case of hydrocephaly were found in the historical control database (13 studies within 4 years of the current study). As no case of hydrocephaly or anophthalmia was observed at the top dose in F1b and no cases were observed at any dose level in F1a or F2, this isolated occurrence is not considered to be related to treatment.

<u>Developmental neurotoxicity study in rats (2006)</u>

The study was conducted according to US EPA OPPTS 870.6300 and under GLP. Pregnant Wistar rats were administered mepiquat chloride via gavage at dose levels of 0, 15, 30 or 60 mg/kg bw/d from GD 6 to PND 10. From PND 11 to PND 21 the pups were dosed directly at the same dose levels. Top dose selection was based on preliminary studies where clinical signs (tremors, lateral position) and mortality were observed from 200 and 75 mg/kg bw/d in dams and pups respectively.

The top dose in the main study (60 mg/kg bw/d) was not toxic to dams but caused increased mortality of pups mostly within the first few days of dosing (out of 224 dosed pups, 15 pups died during PND 11-14, 7 pups died during PND 15-21). A follow-up acute toxicity study in pre-weaning pups (2006), described in the acute toxicity section, indicates that this pup mortality represents acute toxicity rather than a developmental effect.

Brain morphometry on PND 62 showed statistically significant decreases in the size of certain brain regions in females (corpus callosum by 14%, hippocampus left by 6%, folia pyrimidus of the cerebellum by 7%). The differences were no longer significant after Bonferroni-Holm correction for multiple comparisons. All values remained within the HCD range (7 studies, date of studies not specified). No other developmental effects were found in this study.

Developmental study in rats (1977)

In this non-guideline and pre-GLP study mepiquat chloride was administered to pregnant Sprague-Dawley rats at dietary concentrations up to 3000 ppm (purity not specified) on GD 0-20 (25/group) or GD 0 – PND 21 (10/group). No significant maternal or developmental toxicity was observed in this study.

Conclusion on classification for development

RAC has identified two findings potentially relevant for classification:

- Strong developmental retardation in the 2-generation study (1993) and the 1-generation range-finding study
- Hydrocephaly in the rabbit PNDT study (1998)

As the developmental retardation in dietary generational studies occurred exclusively at maternally toxic doses, this effect is likely to represent a secondary, non-specific consequence of maternal toxicity, and as such is of low relevance for classification.

Hydrocephaly in the rabbit study was limited to one litter per dose level (litter incidence 0, 1, 0 and 1 at 0, 50, 100 and 150 mg/kg bw/d respectively; foetal incidence 0, 1, 0, 2). Historical control database reported a single case in 10 studies. There is some uncertainty as to whether the few cases of hydrocephaly in this study could be related to treatment. RAC notes that the two affected foetuses (at the top dose) come from the same litter, which makes the dose-response relationship rather weak. Therefore, RAC does not consider the evidence of developmental toxicity from this study sufficiently strong to trigger classification.

Contrary to the DS's reasoning, RAC does not find evidence of a treatment-related increase in anophthalmia, and the low incidence of hydrocephaly in the rabbit PNDT study without a clear dose-response relationship is not considered sufficient for classification. The developmental retardation in the 2-generation study is likely to be secondary to maternal toxicity. Therefore, RAC proposes no classification for development.

***Adverse effects on or via lactation***

The only finding potentially attributable to an effect on or via lactation is a strong developmental delay in the generational studies (2-generation study, 1993, and a corresponding range-finding study). However, this finding is likely to represent a secondary, non-specific consequence of maternal toxicity as discussed in the section on development. There is no information on excretion via milk. RAC proposes no classification for effects on or via lactation.

***Overall conclusion on reproductive toxicity***

RAC is of the opinion that **classification of mepiquat chloride for reproductive toxicity is not warranted**.

## Supplemental information - In depth analyses by RAC

Range-finding study to the 2-generation study (1993)

This summary is based on information from the study report of the main study. Wistar rats (10/sex/group) were administered mepiquat chloride at dietary concentrations of 0, 2000,

4000 and 6000 ppm (equivalent to about 0, 200, 400 and 600 mg/kg bw/d during premating and gestation and 0, 380, 700 and 1000 mg/kg bw/d during lactation). After an about 6-week rearing period the animals were mated. The F1 generation pups derived from this mating were reared until about PND 21 and then killed.

Selected findings in parental animals at 6000 ppm:

- Reduced food consumption (premating by 15%/13% m/f)
- Reduced body weight, in females by 12% (premating), 14% (gestation) and 21% (lactation) compared to control; in males by 11% (premating)
- Tremor and hypersensitivity in all dams during lactation

Reproductive findings and findings in pups at 6000 ppm:

- Reduced number of pups per dam
- Reduced pup weights (on PND 21 a 33% reduction compared to control), significantly retarded growth of pups

Selected findings in parental animals at 4000 ppm:

- Reduced body weight gain
- Tremor in 9/10 dams and hypersensitivity in 2/10 dams during lactation

Reproductive findings and findings in pups at 4000 ppm:

- Reduced pup weights (on PND 21 a 17% reduction compared to control), significantly retarded growth of pups

No substance-related findings were observed at 2000 ppm.

<u>4-week dietary study in rats (1992)</u>

Wistar rats (5/sex/group, age 6 weeks) were administered mepiquat chloride at dietary concentrations of 0, 500, 2000 and 8000 ppm (equivalent to ca. 0, 46, 180 and 660 mg/kg bw/d). No mortality or clinical signs of toxicity were observed in this study. Food consumption and body weight became markedly lower than in controls during the first week (top dose females: fc days 1-7 reduced by 36%, bw day 7 reduced by 18% compared to controls). Food consumption in top dose animals improved over the following weeks, the body weight reduction diminished in females but persisted in males.

<u>90-day dietary studies in rats (1992)</u>

In the first 90-day study Wistar rats (10/sex/group) were administered mepiquat chloride at dietary concentrations up to 4630 ppm (equivalent to ca. 350 mg/kg bw/d). The originally intended top dose was 8000 ppm but there was a measurement error in making the test diet with a correction for mepiquat chloride content of 58% in the aqueous solution. No toxic effects were observed in this study except a transient initial decrease in food consumption and body weight gain in top dose males. Therefore, a supplementary 90-day study was conducted, testing a dietary concentration of 12000 ppm.

In this second 90-day study the animals (10/sex/group, age 6 weeks) were administered mepiquat chloride at dietary concentrations 0 and 12000 ppm (equivalent to ca. 830 mg/kg bw/d in males and 950 mg/kg bw/d in females). This dose level proved to be above MTD for this type of study. Food consumption over the first week was reduced by 66%/55% (m/f), and over the study period by 31%/18% (m/f). After an initial body weight loss, the top dose animals began to gain weight but the body weights remained lower by about 33%/17% (m/f) compared to controls throughout the study period. Tremor started in

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| several animals in the second week with almost all animals affected by week 5. Most animals also showed abnormal gait and/or abdominal position, especially in the second half of the study. No animal died prematurely. |

## 10.11 Specific target organ toxicity-single exposure

**Table 61: 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** |
|---|---|---|---|
| Acute oral neurotoxicity study<br>OECD TG 424 (1997), GLP<br>Food consumption was not measured. When there was microscopical findings on high dose group, these tissues were not examiced microscopically in mid and low dose groups.<br>Rat, Wistar<br>10/sex/group<br>Acceptable<br>Key study | Mepiquat chloride batch no. 2000-1<br>Purity: 617.6 g/L<br>0, 100, 300, 1200 mg/kg bw/day corresponding to active ingredient doses (a.i.) 0, 58, 174, and 697 mg/kg bw<br>Single administration by gavage | 174 mg/kg bw: statistically significantly reduced motor activity in males.<br>697 mg/kg bw: one male died, males had statistically significantly reduced body weights gains. Both sexes had statistically significantly reduced motor activity and rearing on the day of administration. Both sexes exhibited findings on functional observation battery on the day of administration e.g. squatting posture, respiration labored, tremors and impairment of coordination.<br>No mortality in females | dRAR B.6.7.1.<br>2002, 2003 |
| Acute oral toxicity study<br>OECD TG 401 (1987)<br>Rat, Wistar<br>5/sex/dose<br>Acceptable | Mepiquat chloride batch no. WW 262/CP1490<br>Purity: 57.9 % with 44.3 % water<br>100, 200, 464, 1470, 2150 mg/kg bw corresponding to active ingredient doses (a.i.) 58, 115, 270, 851, 1245 mg/kg bw<br>Single administration by gavage | Clinical signs including poor general state, dyspnea, apathy, abdominal position, staggering, twitching, compulsary gnawing and cyanosis were observed up to 1 hour after administration at dose levels of ≥ 270 mg/kg bw in both sexes. No signs of toxicity were observed at dose levels of ≤ 115 mg/kg bw.<br>$LD_{50}$: males 270 mg/kg bw, females 115 - 270 mg/kg bw | dRAR B.6.2.1.<br>1989 |

| | | | |
|---|---|---|---|
| Acute oral toxicity study<br>OECD TG 401 (1987)<br>Mouse, NMRI<br>5/sex/dose<br>Acceptable | Mepiquat chloride batch no.:WW 262/CP1490,<br>Purity 57.9 %<br>100, 200, 464, 1470, 2150 mg/kg bw corresponding to active ingredient doses 58, 115, 270, 851, 1245 mg/kg<br>Single administration by gavage | At ≥ 270 mg/bw kg mortalities and clinical signs of toxicity including dyspnoea, apathy, abdominal position, lateral position, staggering, twitching, clonic convulsions, exsiccosis in both sexes.<br>Additionally tremor, piloerection and weight reduction were observed in females in the 1245 mg/kg bw dose group.<br>$LD_{50}$ both sexes: 450 mg/kg bw | dRAR B.6.2.1.<br>1989 |
| Acute inhalation toxicity<br>OECD TG 403<br>(fulfills mainly 2009)<br>Rat, Wistar<br>5/sex/dose<br>Acceptable | Mepiquat chloride batch no.:WW 262/CP1490<br>Purity not stated, but it is presumably 57.9 % based on the batch used.<br>liquid (water) aerosol<br>2.59 and 4.89 mg/L, corresponding to a.i. doses 1.50 mg/L and 2.84 mg/L<br>particle size: 2.7 µm - 2.9 µm | At 1.50 mg/l during the exposure irregular, accelerated and intermittent respiration, eyelid closure, ruffled fur. The signs were reversible in 48 hours.<br>At 2.84 mg/l irregular, accelerated and gasping respiration, abdominal, lateral or squatting position, tonic-clonic convulsion, eyelid closure, ruffled fur. One male and 2 females of the 2.84 mg/l dose group died within 24 h after dosing. After day 6 all survivors were without findings<br>$LD_{50}$ both sexes: ≥2.84 mg/L | dRAR B.6.2.3.<br>1991 |
| Acute dermal toxicity<br>OECD TG 402 (1987)<br>Rat, Wistar<br>5/sex/dose<br>Inconclusive due to too low dose | Mepiquat chloride batch no.:WW 262/CP1490<br>Purity 57.9 %<br>2000 mg/kg bw in water corresponding to 1160 mg a.i./kg bw | Both sexes:<br>No mortality, clinical signs of toxicity or local reaction to treatment were observed.<br>$LD_{50}$ > 2000 mg/kg bw, equivalent to >1160 mg/kg bw<br>Data is inconclusive for classification since dose higher than 1160 mg/kg bw was not used | dRAR B.6.2.2.<br>1989 |

**Table 62: Summary table of other studies relevant for STOT-SE**

| Type of study/data | Test substance | Observations | Reference |
|---|---|---|---|
| *In vitro* testing on nicotinic acetylcholine receptors activity of adult mouse muscle (patch clamp technique)<br>No TG, no GLP<br>Acceptable | Mepiquat chloride<br>Batch no.: WW 285<br>Purity: 99 % | Single channel openings elicited by binding of mepiquat chloride to the nicotinic receptor could be detected in all experiments. It was reported that the effect of 1000 µM mepiquat chloride was the same as the effect of 10 µM acetylcholine and mepiquat chloride must therefore be considered as a partial agonist of nicotinic Acetylcholine activated receptors (AChR) *in vitro*. | dRAR B.6.8.2, 1991 |
| *In vitro* testing on the affinity of Mepiquat chloride for muscarinic receptors.<br>No TG, no GLP<br>Acceptable | Mepiquat chloride<br>Batch no.: WW 285<br>Purity: 99 % | Mepiquat chloride was shown to have low affinity to muscarinic AChR in vitro. $K_i$- values of mepiquat chloride were about 5 orders of magnitude higher than those of atropine. It was concluded that mepiquat chloride has measurable though very low and rather unselective affinity to muscarinic receptors. | dRAR B.6.8.2., 1991 |
| Subchronic oral neurotoxicity study<br>OECD TG 424 (1997)<br>GLP<br>Rat, Wistar<br>10/sex/dose<br>Acceptable | Mepiquat chloride<br>Batch no.:2000-1,<br>Purity: 617.6g/L<br>0, 1625, 6500, 13000 ppm, corresponding to achieved dietary intakes 65.6, 259.0, 516.6 mg/kg bw/day and 79.4, 366.9 and 616.5 mg /kg bw/day in males and females, respectively | No mortalities or clinical signs of toxicity.<br>259/366.9 mg/kg bw/day:<br>Slightly reduced body weight gain (8.6 % in males and 10.9 % in females)<br>516.6/616.5 mg/kg bw/day:<br>Markedly reduced body wight gain (28.1 % in males and 29.0 % in females).<br>Reduced grip strength of hindlimbs in females (statistical significance on day 85) and reduced grip strength of forelimbs in males (statistical significance on day 22). Occasional decrease of mean motor activity of males. Statistically significant decreases in rearing in high and low dose males on day 22. | dRAR B.6.7.1.,2002, 2002, 2003 |
| Developmental neurotoxicity study rat<br>OECD TG 426, with some deviations, GLP<br>10 pups/sex/dose were examined with neurological tests<br>Highest dose was too low for dams | Mepiquat chloride<br>0, 15, 30, 60 mg /kg bw/day<br>oral administration via gavage: dams days 6 p.c.-10 p.p., pups days 11 p.p.-21 p.p. | Pup systemic toxicity and mortality after direct dosing of pups at 30 and 60 mg/kg bw/day<br>No clear treatment related changes in neurological tests | dRAR B.6.7.1., 2006 |

| | | | |
|---|---|---|---|
| Acute effects in pre-weaning Wistar rats<br>no TG<br>GLP | Mepiquat chloride<br>oral administration via gavage directly to pups<br>0, 30, 60, 120, 200 mg /kg bw/day<br>on days 11-21 post partum (p.p.) | Acute lethality of pups at 60 (4%), 120 (55%) and 200 mg/kg bw/day (100%). Tremors and lateral position was observed in pups of 120 mg/kg bw/day group 2-6 hours after administration. | dRAR B.6.7.1., 2006 |
| 2-generation study<br>OECD TG 416 (2001)<br>GLP<br>Wistar rats<br>Acceptable | Mepiquat chloride<br>0, 500, 1500, 5000 ppm | Reduced grip strength of the forelimbs and hindlimbs in high dose parental animals. Tremor and hypersensitivity in high dose (5000 ppm corresponding to 500 mg/kg bw/day) dams | dRAR B.6.6.1., 1993 |
| Supplementary study on the oral toxicity of Mepiquat chloride in Wistar rats. | Mepiquat chloride<br>oral in a diet over 3 months<br>12 000 ppm, corresponding to 826 and 951 mg/kg bw/day in males and females, respectively | 826/951 mg/kg bw/day:<br>Impaired behaviour, tremors, impaired gait, ataxia, posture abnormalities and corneal clouding.<br>Reduction in grip strength in both sexes, increased values in hot plate test in males<br>No lethality. | dRAR B 6.3.2., 1992 |

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

Specific target organ toxicity after single exposure of mepiquat chloride has been studied in one acute oral neurotoxicity study in rat (dRAR B.6.7.1., 2002, 2003), in two oral acute toxicity studies (rat and mice, dRAR B.6.2.1., 1989) and in acute toxicity studies via inhalation and dermal routes (dRAR B.6.2.3., 1991 and B.6.2.2., 1989). The studies generally comply with older OECD test guidelines (TG 424 1997, TG 401-402 1987) and have been conducted in accordance with GLP. The studies are only briefly described below. Further details are given in dRAR and for acute toxicity studies also in the sections 10.1.- 10.3. of this CLH report.

**In acute oral neurotoxicity study** (dRAR B.6.7.1. 2002 and 2003) groups of 10 male and 10 female Wistar rats were administered by gavage mepiquat chloride in distilled water as a single oral dose with active ingredient (a.i.) doses of 0, 58, 174, and 697 mg/kg bw. The animals were observed for up to 2 weeks after dosing. Functional observation battery of tests (FOB) and motor activity measurements were carried out in all animals prior to the test substance administration (day -7), on the day of administration (day 0) and 7 and 14 days after dosing. FOBs were performed on day 0 about 2 hours after administration of mepiquat chloride and motor activity measurements were conducted each timepoint after the FOBs. At termination of the study (day 15), 5 animals/sex/dose group were sacrificed by perfusion fixation. The sacrificed animals were necropsied and the visible organs assessed by gross pathology. Comprehensive range of tissue samples from central and periferous nervous systems were processed histochemically (see dRAR for details). Control and high dose groups specimens were examined microscopically, the specimens of low and mid dose groups were preserved.

One high dose male (697 mg/kg bw) died on the day of substance administration (day 0). This was assessed as being treatment related. One high dose animal showed piloerection from day 1-3. The body weight gain of high dose males was statistically significantly decreased compared to controls on day 7.

**Table 63: Body weight gain (g) in acute neurotoxicity study**

| | **0** | **58 mg/kg bw** | **174 mg/kg bw** | **697 mg/kg bw** |
|---|---|---|---|---|
| **Males** | | | | |
| Day 7 | 40.6 | 37.0 | 38.6 | 31.5*<br>78% of control |
| Day 14 | 74.7 | 65.6<br>88% of control | 70.8<br>95% of control | 64.1<br>86% of control |
| **Females** | | | | |
| Day 7 | 14.8 | 15.7 | 16.8 | 16.0 |
| Day 14 | 29.3 | 28.9 | 30.2 | 29.3 |

*Statistically significance at P< 0.01 compared to control

On the day of the substance administration (day 0) high dose (697 mg/kg bw) animals exhibited a range of effects in FOB tests (see dRAR for detailed data). These effects included abdominal position (3/10 and 5/10 males and females respectively), eyelids half closure (6/10 and 2/10 male and females, respectively), squatting posture (3/10 and 5/10), respiration labored or gasping/ respiratory sounds (7/10 and 4/10), slight or moderate tremors (7/10 and 4/10), slight or moderate impairment of coordination (7/10 and 6/10), animal was unable to walk (1/10 male), reduced exploration of the area or severe reduced exploration of the area (8/10 and 6/10), piloerection (1/10 male), pupillary reflex/no response to the stimulus (6/10 and 6/10). The effects resolved by day 7. These findings were not reported in other groups in any of the time points.

Moreover, motor activity was statistically significantly reduced in high dose males and females compared to controls on day 0 (by 89% and 77% of control level in high dose males and females, respectively, dRAR). Motor activity of mid dose (174 mg/kg bw) males was also statistically significantly lower than controls (only intervals 1 and 2 of bean interrupts). The study reporter stated that it could not be excluded that this effect was treatment-related. Rearing was also statistically significantly reduced in high dose males and females on day 0. Statistically significantly increased rearing was reported in mid dose (174 mg/kg bw) males on day 0 but this was assessed as being incidental. Grip strength of hindlimbs was statistically significantly decreased in high dose females on day 14. Due to the late occurrence and slightness of the effect this was assessed as being incidental and not treatment-related. No significant differences between the groups were observed in grip strength of forelimbs or food spay test.

**Table 64: Results of rearing test**

| | **Control** | **58 mg a.i. /kg bw** | **174 mg a.i. /kg bw** | **697 mg a.i. /kg bw** |
|---|---|---|---|---|
| **Males** | | | | |
| **Day -7** | 3.5 | 3.9 | 4.7 | 4.0 |
| **Day 0** | 1.6 | 3.1 | 3.8* | 0.2** |
| **Day 7** | 3.2 | 2.5 | 2.0 | 3.7 |
| **Day 14** | 3.6 | 2.4 | 3.7 | 3.0 |
| **Females** | | | | |
| **Day -7** | 10.2 | 8.6 | 10.2 | 9.5 |
| **Day 0** | 10.1 | 9.8 | 9.3 | 0.1** |

| | | | | |
|---|---|---|---|---|
| **Day 7** | 12.8 | 11.0 | 9.3 | 11.7 |
| **Day 14** | 13.4 | 12.5 | 13.3 | 13.0 |

*=0.05, **=0.01

In necropsy, no gross lesions were found on examined animals. In histopathological examination, few cases of minimal (grade 1) axonal degeneration of peripheral nerve fibers (proximal sciatic nerve and distal tibial nerve) were reported in control and high dose groups (dRAR, incidences sciatic nerve 2, 0,0,0 and 0, 0, 0, 2 at 0, 58, 174, 697 mg/kg bw in males and females, respectively and distal tibial nerve 2, 0, 0, 1 and 0, 0, 0, 1 at 0, 58, 174, 697 mg/kg bw in males and females, respectively). There were no remarkable differences in histological findings between groups.

In dRAR the NOAEL of the study was set 174 mg/kg bw based on findings on males and females in functional observation battery on day 0 and on statistically significantly decreased rearing and motor activity on males and females on day 0 at 697 mg/kg bw. However, in the study report NOAEL for males was set 58 mg/kg bw based on slight, but statistically significantly reduced motor activity at 174 mg/kg bw.

In the study report it was stated that the clinical effects observed were explained by reactivity of the test substance with nicotinergic and muscarinergic receptors and represents reversible binding to receptors rather than irreversible neurotoxicity.

**In rat acute oral toxicity study** (dRAR B.6.2.1., 1989) groups of 5 male and 5 female Wistar rats were administered by gavage a single oral dose of mepiquat chloride with active ingredient doses 58, 115, 270, 851, 1245 mg/kg bw. Observation period was 14 days for doses 58 to 270 mg bw /kg and 0 days for 851 and 1245 mg/kg. Signs and symptoms were recorded several times on the day of administration, and at least once each workday. Mortalities were checked twice on each workday. Necropsies, with gross-pathological examination, were performed on fasted animals.
There were no mortalities or signs of toxicity at dose levels 58 and 115 mg/kg bw. Clinical signs including poor general state, dyspnea, apathy, abdominal position, staggering, twitching, compulsary gnawing and cyanosis were observed up to 1 hour after administration at dose levels of ≥ 270 mg/kg bw in both sexes. At 270 mg/kg bw two males and three females were found dead 24 hours after dosing. At 851 and 1245 mg/kg bw all animals died or were sacrificed in moribund condition within 1–24 h after dosing. Mean body weight gains were not significantly affected by the treatment. Gross examination at necropsy revealed general congestion in animals which died but no abnormal observations were made in survivors killed at scheduled termination. The acute oral $LD_{50}$ of mepiquat chloride in the rat was approximately 270 mg/kg bw for males and 115 – 270 mg/kg bw in females.

I**n mouse acute oral toxicity study** (dRAR B.6.2.1., 1989) groups of 5 male and 5 female NMRI mice were administered by gavage a single oral dose of mepiquat chloride with active ingredient doses 58, 115, 270, 851, 1245 mg/kg bw. Observation period was 14 days. Signs and symptoms were recorded several times on the day of administration, and at least once each workday. Necropsies, with gross-pathological examination, were performed on fasted animals.
Mortalities occurred at dose levels of ≥ 270 mg/kg bw within 1h after dosing. Clinical signs at ≥ 270 mg/kg bw in both sexes included poor general state, dyspnoea, apathy, abdominal position, lateral position, staggering, twitching, clonic convulsions, exsiccosis. Additionally tremor, piloerection and weight reduction were observed in females in the 1245 mg/kg bw dose group. Most of the symptoms were reversible within 4 hours except for weight reduction which was observed until day 13 in survivors. No clinical signs of toxicity were reported at dose levels 58 and 115 mg/kg bw. Gross examination at necropsy revealed general congestion in animals which died but no pathological findings were noted in survivors killed at scheduled termination. According to study report the acute oral $LD_{50}$ of mepiquat chloride in mice was approximately 450 mg/kg bw for both sexes.

**In rat acute inhalation toxicity study** (dRAR B.6.2.3., 1991) groups of 5 male and 5 female Wistar (SPF Wistar/Chbb) rats were exposed (head-nose) for four hours to an aerosol of mepiquat chloride 1.50 or 2.84 mg/L and were thereafter observed for 14 days. One male rat and 2 female rats of the high dose (2.84 mg/L) group died within 24 h after dosing. Clinical findings in the high dose group included irregular, accelerated,

intermittent respiration (5/10, 10/10 and 4/10 animals, respectively) gasping (4/10 animals) and eyelid closure (10/10). Attempts to escape were noted during the first 15 minutes of exposure (10/10). Animals that died spontaneously showed general congestion and in lungs focal hyperaemia. In the low dose (1.50 mg/L) group clinical findings observed during the exposure period included irregular, accelerated and intermittent respiration in some animals (8/10, 4/10 and 2/10 animals, respectively) and eyelid closure in all animals. After exposure and during the observation period also ruffled fur was observed in all animals in addition to accelerated and intermittent respiration of some animals. 48 hours after exposure all animals of the low dose group were without findings. There was no mortality in the low dose group. In the high dose group, accelerated respiration was seen until day 5 after exposure. Other signs of toxicity were intermittent respiration, respiratory sounds, abdominal, lateral or squatting position in individual animals, tonic-clonic convulsions and discoloured fur with test substance and ruffled fur (all survivors). After day 6 all survivors were without findings. Necropsy on survivors at termination of the study revealed no pathological findings.

**In rat dermal acute toxicity study** (dRAR B.6.2.2., 1989) a dose of 1160 mg/kg bw mepiquat chloride was applied under a semi-occlusive dressing to the clipped dorsal and dorsolateral skin of five male and five female Wistar rats for 24 h. Mortality and signs of toxicity were recorded during the 14-day observation period. There were no deaths and no clinical signs of toxicity or local reaction to treatment were observed.

Other relevant studies

There **are two *in vitro* studies** available in which the receptor binding of mepiquat chloride has been examined (dRAR B.6.8.2, 1991 and 1991). Based on these studies mepiquat chloride is considered as a partial agonist of nicotinic acetylcholine receptor (nAChR) and it also has low affinity to muscarinic acetylcholine receptors.

In addition to in response to single dose administration, signs of neurotoxicity have also been reported in the following repeated dose toxicity studies with mepiquat chloride (see dRAR for details).

**In the rat subchronic oral (dietary) neurotoxicity** study (dRAR B.6.7.1., 2002, 2003) the grip strength of hindlimbs of females was decreased, at the high dose (616.5 mg/kg bw/day) reaching statistical significance at the last observation point on day 85 (30% decrease compared to control). In high dose (516.6 mg/kg bw/day) males the grip strength of forelimbs was statistically significantly decreased by 29% compared to control on day 22. Moreover, the mean motor activity was occasionally decreased on males when compared to controls and rearing was statistically significantly decreases in high and low dose males on day 22. In high dose males, moderate (grade 3) multifocal muscle fiber degeneration with a reactive myositis in the gastrocnemius muscle and an incidence of axonal degeneration (grade 1) in peripheral nerves as a single occurrence in the proximal sciatic nerve and in the proximal tibial nerve (grade 1) were observed in histopathological examination. No mortalities or clinical signs of toxicity were reported.

The reduction in grip strength (both forelimbs and hindlimbs) was also observed in a **3-months feeding study in rats** (dRAR B.6.3.2., 1992) at the only dose level used, 12000 ppm (826/951 mg/kg bw/day). For females the values were reduced on all examination points (days 34, 69, 93), for males on the two first observation points. Also increased values in hot plate test was observed in males on all examination points (days 34, 69, 93). There were no mortalities during the study. Clinical signs of toxicity included reduced general state of health, impaired behaviour, tremors, impaired gait, ataxia, posture abnormalities and corneal clouding. In general, most of the observed clinical findings started during the first weeks of the study and lasted until the end of the test.

Functional observation battery (FOB) was studied also **in a chronic toxicity study in rats** (administration via a diet) (dRAR B.6.5.1., 1994). There were no effects in the neurological examinations at any time point and the grip strength of hindlimbs was not decreased in either males or females. Highest dose level used (achieved daily intake) was 268/371 mg/kg bw/day.

**In the two generation study in rat** (dRAR B.6.6.1., 1993) the examination of the neural function was carried out in all F0 and F1 parental animals before the beginning of the mating period and in parental dams during the lactation period and after weaning of the pups and at about the same time in parental males. Similar effects were observed in parental animals of both generations. Tremor and hypersensitivity were observed in most

high dose (5000 ppm via diet corresponding to 520 mg/kg bw/day) F0 and F1 dams and ataxia in F0 dams during lactation period. In parental F1 males no clinical signs attributed to the test substance were detected.

Grip strength of the forelimbs was statistically significantly reduced in high dose F0 and F1 dams during lactation, in F0 dams also after weaning and in F1 males before mating. Grip strength of hindlimbs was statistically significantly reduced and F1 dams during lactation and F1 males before mating (see dRAR). In neurofunctional tests no abnormalities were detected for both sexes. The hot-plate test values did not show statistically significant differences between treated and control groups.

**The developmental neurotoxicity study** (dRAR B.6.7.1., 2006) is not considered appropriate to examine fetal development or developmental neurotoxicity since the highest dose (60 mg/kg bw/day) was too low for dams. Pups were exposed to the substance at doses 15, 30 and 60 a.i. mg/kg bw/day during pre-, peri- and postnatal development (dams were administrated via gavage from day 6 p.c. to day 10 p.p. and pups directly via gavage from day 10 p.p. to day 21 p.p.). In addition to neurological observation battery and tests included in OECD TG 426, nicotine probe study was conducted on pups to detect potential effect of mepiquat chloride on the nicotine receptor. In auditory startle test the startle maximum amplitude was decreased in male pups of all dose groups compared to control on day 24 p.p.. Also the startle time to peak amplitude was decreased in males at all dose levels on day 24 p.p. and 60 p.p. However, according to study report this was due to high average maximum amplitude of the controls exceeding the historical control range, due to one control male with unexceptionally high value. In the water maze test the mean time to escape was increased in high dose males in memory test day 22 p.p. and in learning test day 60 p.p. and on high dose females on learning test day 22 p.p. However, these effects were not considered treatment-related by the study rappourter. In the nicotine probe study conducted on day 71±4 no treatment-related effects were observed.

In this study acute mortality of pups was observed after the start of direct dosing of pups at mid (30 mg/kg bw/day) and high dose groups (60 mg/kg bw/day). According to dRAR the dose selections for this study were based on the preliminary studies which revealed clinical signs such as tremors, lateral position and lethality at doses 200 and 300 mg a.i./kg bw/day in dams but not at 50 mg a.i./kg bw/day. After direct gavage dosing of pups (days 11-21 post partum) lethality and clinical signs (tremors, lateral position) were observed in at doses of 75-300 mg/kg bw/day. The peak incidence of dams exhibiting tremors and pups found dead occurred 2-3 hours after administration via gavage.

**The study of acute effects in pre-weaning rats** (dRAR B.6.7.1., 2006) mortality of pups administered with mepiquat chloride via gavage on days 11-21 p.p. was observed in all dose groups (60, 120 and 200 mg/kg bw/day) except the low dose group (30 mg/kg bw/day). Deaths occurred approximately 2-4 hours after the administration on days 11-16 p.p. Lateral position (2-6 h after treatment) and tremors (2-6 h after treatment) were observed on at 120 mg/kg bw group.

**In the 3-months study in dogs** (dRAR B.6.3.2.,1977) the main sign of toxicity observed was sedation at 3000 ppm (95.3 mg/kg bw/day) which occurred from the start of treatment for up to 4 weeks and reappeared on individual study days in 3/8 dogs. Maximum signs of sedation were observed from 3 – 8 days after the start of treatment. Signs of sedation were accompanied by abdominal and periodically lateral position and tono-clonic spasms. Based on the information at the study report the effect started on all affected animals from the 1$^{st}$ day onwards about 30 minutes after the feeding. There were no mortality in the study.

**In the 12-months study in dogs** (dRAR B.6.3.2., 1994) the highest dose was initially 8000 ppm. Due to three unexpected deaths (1/6 males and 2/6 females) on day one, the administration of the test substance was discontinued and the surviving animals were allowed to recover for 5 days. Thereafter the death animals were replaced and the study was continued using 6000 ppm (166/173 mg/kg bw/day) as the highest dose. The main sign of toxicity was salivation observed both in males and females at this dose level (6000 ppm). It was reported that salivation was observed from 2 hours after feeding onwards, starting with slight degree after 2 hours and moderate to severe degree until 4-6 hours. Findings were reported to be reversible until the next day prior to feeding. One female dog receiving 6000 ppm was sacrificed in moribund condition during the study.

**In the 28-day study in dogs** (dRAR B.6.3.2., 1994) salivation of varying intensity and frequency was observed in all dogs at 6000 ppm (185 mg/kg bw) and 12000 ppm (308 mg/kg bw) except for one female at 6000 ppm. It was reported that in the male animals salivation was observed in general from 2 hours after feeding onwards until 4 to 6 hours. All findings were reported to be reversible until next day prior to feeding. In females

salivation was observed only sporadically. In the study report the authors stated that activation of the muscarinic receptors *in vivo* may cause salivation. One female dog from the high dose group died on the first day of the study. The rappourter of the study considered this as treatment-related.

### 10.11.2 Comparison with the CLP criteria

Classification as either STOT-SE 1 or 2 is applicable to substances that have produced non-lethal 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 non-lethal toxicity in humans following a single exposure.

Classification as STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

Clear signs of neurotoxicity were reported in rats and mice in response to single mepiquat chloride doses ≥ 270 mg/kg bw by oral route and ≥ 1.50 mg/L by inhalation. These signs included for example squatting posture, irreregular and accelerated respiration, gasping, tremors, convulsions, impairment of coordination and eye lid closure. Moreover, statistically significantly decreased rearing was observed in acute neurotoxicity study in rat in both sexes at dose 697 mg/kg bw and reduced motor activity at doses 697 (both sexes) and 174 mg/kg bw (males). In these studies there was no lethality at doses 174 mg/kg bw and 1.50 mg/L. For comparison, the acute oral $LD_{50}$ values for mepiquat chloride in rat are 270 mg/kg and 115 - 270 mg/kg bw for males and females, respectively and in mice 450 mg/kg bw for both sexes. The acute inhalation $LD_{50}$ value was determined to be ≥ 2.84 mg/L for both sexes (section 10.1-10.3.)

Signs of neurotoxicity were also reported in repeated dose toxicity studies. In rats, statistically significantly decreased grip strength of fore- and hindlimbs, reduced motor activity and rearing were reported in dietary studies at doses ranging 520-951 mg/kg bw/day. Clinical signs in these studies incuded impaired behaviour, tremors, impaired gait, ataxia, posture abnormalities, tremor and ataxia but no lethality occurred. In dietary studies in dogs sedation, abdominal and periodically lateral position and tono-clonic spasms at 95.3 mg/kg bw/day and salivation at dose 166-308 mg/kg bw/day were reported. In the rat developmental neurotoxicity study and study on acute affects in pre-weaning rats (dRAR B.6.7.1.2006) the substance was administrated via gavage directly to pups which resulted acute mortality of pups at doses ≥ 60 mg/kg bw/day (in the other study also 30 mg/kg bw/day caused mortality). Moreover, clinical signs such as tremors, lateral position and lethality after gavage administration at doses 200 and 300 mg/kg bw/day were reported in dams. In some repeated dose studies the neurotoxic signs were described as acute effects after dosing.

Based on *in vitro* studies (dRAR B.6.8.2, 1991 and 1991) mepiquat chloride is a partial agonist of nicotinic acetylcholine receptor and it has low affinity to muscarinic acetylcholine receptors. The observed signs of clinical toxicity can be associated with the activation of the nicotinic receptor (tremors, ataxia, lack of motor coordination, decreased motor activity and abnormal posture), whereas some clinical observations (bradypnea and salivation) may be associated with the activation of the muscarinic receptor.

Classification for STOT-SE based on neurotoxic effects should be considered for mepiquat chloride.

According to CLP criteria: *"specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed STOT-SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality…" Care must be taken not to classify for STOT-SE for effects which are not yet lethal at a certain dose, but would lead to lethality within the numeric classification criteria. In other words, if lethality would occur at relevant doses then a classification for acute toxicity would take precedence and STOT-SE would not be assigned."*

Signs of neurotoxicity in response to single exposure were also observed at doses were no lethality occurred, i.e irregular and accelerated and intermitted respiration and eye lid closure in acute inhalation toxicity study at 1.50 mg/L and decreased motor activity of males in acute neurotoxicity study at 174 mg/kg bw. Moreover, in acute neurotoxicity study only one animal (male) died at high dose (697 mg/kg bw) although a variety of

clinical signs of neurotoxicity were observed both in males and females. On the other hand, the nonlethal dose levels where signs of neurotoxicity occurred (174 mg/kg bw and 1.50 mg/L) lie within the same numeric classification criteria range than $LD_{50}$ and and $LC_{50}$ values for the substance, i.e 50-300 mg/kg bw (Acute Tox 3) and 1-5 mg/L (Acute Tox 4). Therefore we consider the neurotoxic effects as borderline between no classification and classification for STOT-SE but still sufficient to warrant classification.

Although some neurotoxic signs were observed at dose below the guidance value for STOT-SE Category 1 (300 mg/kg bw, reduced motor activity of males at 174 mg/kg bw in acute neurotoxicity study), effects primarily occurred within the range of guidance values for STOT-SE Category 2 (300-2000 mg/kg and 1.0-5.0 mg/l). Thus, classification for STOT-SE 2 for nervous system is proposed.

Signs of neurotoxicity were observed in response to acute oral and inahalation exposure but not after acute dermal exposure (1160 mg/kg bw). Based on study in rats dermal absorption of mepiquat chloride appers to be low (app. 1-3 %, dRAR B.6.1.2., 2003). However, since higher dose than 1160 mg/kg bw has not been tested it is considered that effects after dermal esposure can not be completely excluded. Therefore route of exposure is not proposed to be specified with classification.

Classification for STOT-SE 3 is not warranted, as no signs of respiratory tract irritation were observed in the acute studies available, and the observed neurotoxicity, though transient in nature, does not fulfil the criteria for narcotic effects.

### 10.11.3 Conclusion on classification and labelling for STOT-SE

Based on signs of neurotoxicity observed consistently at both lethal and nonlethal doses, classification for STOT-SE 2; H371 (nervous system) is proposed.

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

### Summary of the Dossier Submitter's proposal

The DS proposed classification with STOT SE 2; H371 (nervous system) based on signs of neurotoxicity in rat, dog and mouse studies at lethal and non-lethal doses. The DS acknowledged that some of the effects may be covered by the proposed acute toxicity classifications and considered the case borderline with no classification.

### Comments received during consultation

Comments were received from 2 MSCAs and a manufacturer. The commenting MSCAs supported the DS's proposal whereas the manufacturer did not consider the effects listed by the DS sufficient for a STOT SE classification. The manufacturer's argumentation can be summarized as follows:

- Clinical signs observed at lethal doses are addressed by the proposed acute toxicity classifications (Acute Tox. 3 for the oral route, Acute Tox. 4 for the inhalation route).
- Some of the clinical signs at peri-lethal doses in acute studies, such as reduced activity at the top dose in the acute neurotoxicity study, reflect generalised toxicity rather than being specific indications of neurotoxicity.

- Clinical signs in repeat dose studies occurred only at doses with high systemic toxicity as indicated for example by markedly impaired body weight development. Moreover, they do not represent effects after a single exposure.
- Sedation reported in an old dog study (from 1977) was not reproduced in more recent studies using higher doses. Salivation reported in the more recent dog studies occurred at doses associated with mortality and does not in itself represent significant or severe toxicity.

## Assessment and comparison with the classification criteria

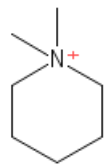
The table below summarizes findings potentially relevant findings for a STOT SE classification at non-lethal and lethal doses.

| **Findings potentially relevant for a STOT SE classification** | | | |
|---|---|---|---|
| **Study** | **Dose (concentration), non-lethal** | **Clinical signs at non-lethal dose** | **Other findings;**<br>**Symptoms potentially related to neurotoxicity at lethal doses** |
| ***Oral gavage*** | | | |
| Acute toxicity, rat (1989) | 120 mg/kg bw | None | 270 mg/kg bw: mortality (5/10), staggering, twitching, compulsive gnawing |
| Acute neurotoxicity, rat (2002) | 170 mg/kg bw | None | FOB: reduced motor activity (males, day 0)<br>700 mg/kg bw: mortality (1/20), lethargy, abdominal position, unsteady gait, tremor, half closure of eyelids, lack of pupillary reflex |
| PNDT, rat (1992) | 300 mg/kg bw/d | Tremor, unsteady gait, piloerection, hypersensitivity (already after the 1st dose, resolved 4 hours post-dosing) | Reduced food consumption and body weight<br>600 mg/kg bw/d (preliminary study): mortality (5/10) after 1-2 doses; tremor, unsteady gait, ataxia, hypersensitivity 1-2 hours after treatment |
| Developmental neurotoxicity, rat (2006) | 60 mg/kg bw/d | Dams: none<br>Pups: increased mortality | 200 mg/kg bw/d (preliminary study), dams: mortality, tremors (peak incidence of tremors 2-3 h after dosing) |
| Acute effects in pre-weaning rats (2006) | 60 mg/kg bw/d | None | 120 mg/kg bw/d: mortality (55%), tremors (peak |

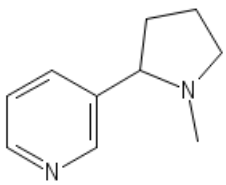
| | | | |
|---|---|---|---|
| | | | incidence of tremors 2-6 h after dosing) |
| Acute toxicity, mouse (1989) | 120 mg/kg bw | None | 270 mg/kg bw: mortality (4/10), staggering |
| Micronucleus test, mouse (2002) | 2x 470 mg/kg bw<br>2x 310 mg/kg bw | Poor general state, squatting posture (after a single dose) | 630 mg/kg bw: mortality (2/5 after 1st administration), poor general state, squatting posture |
| ***Oral dietary*** | | | |
| 4-week, rat (1992) | 660 mg/kg bw/d | None | |
| 3-month, rat (1992) | 890 mg/kg bw/d | Week 1: none<br>Week 2: tremor (7/20)<br>Week 4: tremor (18/20)<br>From week 5: tremor, abnormal gait (long-legged, unsteady), abdominal position | FOB week 5: tremors, ataxia, posture abnormality, reduced grip strength<br>Food consumption: week 1 reduced by ca. 60%, week 5 by ca. 30%/20% m/f<br>Body weight: week 1 bw loss, week 5 bw lower by 34%/17% compared to controls |
| Subchronic neurotoxicity, rat (2002) | 570 mg/kg bw/d | None | FOB: no consistent effects<br>Histopathology: axonal degeneration tibial nerve males 2/5, grade 1 (not considered treatment-related by the study author) |
| 2-generation, rat (1993) | 550 mg/kg bw/d | Tremor and hypersensitivity in lactating dams (intake 630-720 mg/kg bw/d) | FOB: reduced grip strength in lactating dams<br>Reduced body weight and food consumption |
| 3-month, mouse (1992) | 2100 mg/kg bw/d | None | |
| 4-week, dog (1994) | 190 mg/kg bw/d | Salivation 2-6 hours after feeding | 310 mg/kg bw/d: mortality 1/4 on day 1, salivation after feeding |
| 3-month, dog (1977) | 95 mg/kg bw/d | Slight sedation after feeding from the 1st day, maximum effect around the 5th day, then decreasing | |
| 12-month, dog (1994) | 170 mg/kg bw/d | Salivation after feeding | The treatment started with a higher dose (8000 ppm), but due to mortalities |

| | | | |
|---|---|---|---|
| | | Week 3: 1 animal (out of 12) ataxia of hind limbs, lateral position and extension spasm, subnormal body temperature, poor general condition, sacrificed on day 17 | (3/12) on day 1 treatment was discontinued and the dose was reduced to 6000 ppm (170 mg/kg bw/d) |
| ***Inhalation*** | | | |
| Acute toxicity, rat (1991) | 1.5 mg/l | During exposure: irregular respiration, accelerated respiration, eyelid closure<br>After exposure: accelerated respiration, ruffled fur | 2.8 mg/l: mortality (3/10), accelerated/gasping respiration, eyelid closure, squatting position, tonic-clonic convulsions |
| ***Dermal*** | | | |
| Acute toxicity, rat (1989) | 1200 mg/kg bw | None | |
| 4-week, rat (2002) | 1000 mg/kg bw | None | |

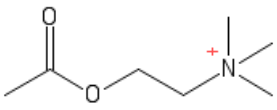
Besides *in vivo* studies, there is a set of *in vitro* studies from 1991 investigating affinity of mepiquat chloride to nicotinic and muscarinic acetylcholine receptors. Mepiquat chloride had a measurable but very low and rather unselective affinity to muscarinic receptors *in vitro*. In the nicotinic receptor study employing adult mouse muscle fibres mepiquat chloride was found to activate the nicotinic acetylcholine receptor channel in all experiments from a concentration of 10 µM. The frequency of channel openings was comparable between 1000 µM mepiquat chloride and 10 µM acetylcholine. The author of the study concluded that mepiquat chloride has to be considered a partial agonist of the nicotinic receptor. They explained that activation of the nAChR will cause depolarization of the muscle fibres and consequently first excitation of the muscle and then muscle weakness. The structure of mepiquat shows some degree of similarity to (but also differences from) nicotine and acetylcholine, see below.




**mepiquat**




**nicotine**




**acetylcholine**

Clinical signs indicative of neurotoxicity (e.g. tremor) were observed in a number of oral studies. They were often limited to a few hours post-dosing; it has been hypothesized that this is due to reversible receptor binding. RAC agrees with the DS that nervous system is

a target organ of mepiquat chloride. The effect was usually observed already after a single or a few doses, so it is considered acute rather than chronic in nature.

Neurotoxicity was observed at or just below lethal doses, which raises a question whether a STOT SE classification for neurotoxicity in addition to an acute toxicity classification would be a 'double classification'. The CLP guidance provides the following advice in this regard:

*"Care must be taken not to classify for STOT-SE for effects which are not yet lethal at a certain dose, but would lead to lethality within the numeric classification criteria. In other words, if lethality would occur at relevant doses then a classification for acute toxicity would take precedence and STOT-SE would not be assigned."* (3.8.2.1.2)

In this case the acute oral toxicity classification is Acute Tox. 3. The respective ATE range is 50 mg/kg bw < ATE ≤ 300 mg/kg bw, the ATE for mepiquat chloride is 270 mg/kg bw. The STOT SE ranges for the oral route are 300 mg/kg bw < C ≤ 2000 mg/kg bw for Category 2, and C ≤ 300 for Category 1.

The rat PNDT study (1992) reported clinical signs of neurotoxicity (tremor, hypersensitivity) without concurrent mortality at 300 mg/kg bw/d. As this dose is above the oral ATE (270 mg/kg bw) obtained with the same species (rat) and way of administration (gavage), RAC agreed that an additional STOT SE classification would represent a double classification and should not be assigned.

The effects at non-lethal doses in dog studies, such as salivation (4-week dog dietary study, 1994, 190 mg/kg bw/d) or sedation (3-month dog dietary study, 1977, 95 mg/kg bw/d) are also indicative of neurotoxicity but their severity is not sufficient for a STOT SE 1 or 2 classification. Sedation in the older dog study (1977) would be consistent with a STOT SE 3 classification for narcotic effects, but no similar clinical signs were seen at a higher dose the more recent studies (1994). Salivation, observed in the 1994 studies, is not suggestive of a narcotic effect.

Overall, RAC does not find in the available studies a sufficiently consistent evidence of effects warranting a STOT SE 3 classification. The more severe neurotoxic effects in some of the rat oral studies are already covered by the proposed acute toxicity classifications. In conclusion, RAC is of the view that a STOT SE **classification is not warranted**.

## 10.12 Specific target organ toxicity-repeated exposure

Not assessed in this dossier

## 10.13 Aspiration hazard

Not assessed in this dossier

# 11 EVALUATION OF ENVIRONMENTAL HAZARDS

## 11.1 Rapid degradability of organic substances

A brief summary of studies considered reliable and relevant on degradation, listed in the Draft Renewal Assessment Report (dRAR), are reported below.

### Table 65: Summary of relevant information on rapid degradability

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Ready biodegradability | | | |
| OECD TG 301 A: Ready Biodegradability: DOC Die-Away (1992)<br><br>Unlabelled mepiquat chloride (chemical purity unknown)<br><br>GLP compliant | **100 %** of mepiquat chloride was **degraded** within **10-day window** during the **28-day test**. | 10 % of degradation was reached in 19 days. After that, the degradation accelerated resulting to 90-100 % degradation. Adsorption of the test item was ruled. Therefore, the substance is **readily biodegradable**. | 2003<br><br>dRAR B.8.3.2.1/01<br><br>**Key study** |
| Hydrolysis | | | |
| US EPA: Pesticide Assessment Guidelines Subdivision N: Section 161-1: Hydrolysis studies (1982)<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 66.748 mBq/mmol and radiochemical purity > 98%)<br><br>GLP compliant | Mepiquat chloride was found to be **hydrolytically stable** at pH 3, 5, 7 and 9 at 25°C during the 30 day test. | The sterility was not checked. However, this deviation is not considered to invalidate the study due to no hydrolysis was observed. | 1989<br><br>dRAR B.8.3.1.1/01 |
| Water, water-sediment and soil degradation data (including simulation studies) | | | |
| Aerobic mineralisation in surface water | | | |
| OECD TG 309: Simulation biodegradation test (2004)<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 6.429 MBq/mg and radiochemical purity 99.2%)<br><br>GLP compliant | Levels of [2,6-$^{14}$C]-mepiquat chloride were found to **remain almost constant** and ranging from 91.6% to 101.1% of AR during the 61-day study period at pH 7.00-7.46 at 20 ± 2°C. | Mepiquat chloride was found to be **stable** or **degrading very slowly** as maximum formation of 0.6% of metabolites were observed. Only small amounts of $^{14}CO_2$ (max formation 4.8% of AR) and other volatiles (maximum formation 0.1% of AR) were detected. Therefore, no degradation kinetics analysis was performed. | 2016<br><br>dRAR B.8.3.2.2/01 |
| Water-sediment data | | | |
| OECD TG 308: Aerobic and anaerobic transformation in aquatic sediment systems (2008) *<br><br>US EPA: Pesticide Assessment Guidelines Subdivision N: Section 162-4: Aerobic aquatic metabolism (1982)<br><br>BBA: Richtlinien für die | Geometric means from kinetic evaluation of the data:<br><br>**Mepiquat chloride $DT_{50}$ and $DT_{90}$ in total systems (SFO kinetics):**<br>Kellmetschweiher Pond: **32 d** ($DT_{50}$) and 106 d ($DT_{90}$)<br>Ranschgraben Stream: **33 d** ($DT_{50}$) and 108 d | Mepiquat chloride mineralized **substantially** in two aquatic systems with sediments. No metabolites could be identified in this study.<br><br>The degradation rates of mepiquat chloride in total system were re-calculated in accordance with FOCUS Degradation Kinetics Report (2006, 2014). | 2002 & 2016<br><br>dRAR B.8.3.2.3/01 & dRAR B.8.3.2.4/01 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Prüfung von Pflanzenschutzmitteln (Nr. IV, 5–1): Abbaubarkeit und Verbleib von Pflanzenschutzmitteln in Wasser/Sediment-System (1990)<br><br>SETAC guideline: Procedures for assessing the environmental-fate and ecotoxicity of pesticides: Part 1: Section 8.2: Aerobic aquatic degradation (1995)<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 1.62 MBq/mgand radiochemical purity > 99%)<br><br>FOCUS Degradation Kinetics Report (2006, 2014)<br><br>GLP compliant | ($DT_{90}$) | Of the kinetic models SFO (Single First-Order) and FOMC (First Order Multi-Compartment) compared, the SFO kinetics gave better predictions (more visually acceptable fit and lower prosentual error to pass $\chi^2$ test). | |
| Soil degradation data | | | |
| Aerobic degradation in soil | | | |
| FOCUS Degradation Kinetics Report (2006, 2014)<br><br>Non GLP | The degradation pathway scheme was derived from the kinetic parameters suitable for modelling the soil degradation of mepiquat chloride.<br><br>As a result, mepiquat chloride was detected degradating $CO_2$ and bound residues through unknown intermediates. | The degradation rates of mepiquat chloride in the three studies below (dRAR B.8.1.1.1/02, B.8.1.1.1/03 & B.8.1.1.1/04) available were re-calculated in accordance with current FOCUS Degradation Kinetics Report (2006, 2014) using KinGUII 2.0 software (Bayer CropScience, 2011).<br><br>A few different kinetic models were fitted to the data in order to find the one that can predict best the experimental results. The models fitted were SFO (Single First-Order), FOMC (First Order Multi-Compartment) and DFOP (Double First-Order in Parallel).<br><br>The suitability of the fit of the models was evaluated both visually and statistically by calculating the minimum prosentual error required to pass the $\chi^2$ test at a probability of 0.05 (acceptability criteria $\chi^2$ error < 15%). | 2016<br><br>dRAR B.8.1.1.1/01 |
| BBA: Richtlinien für die Prüfung von | Non-normalized best fit **(SFO) $DT_{50}$** values at 20 | Mepiquat chloride was found to degrade rapidly at 20°C and only | 2003 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Pflanzenschutzmitteln (Nr. IV, 4–1): Bestimmung des Verbleibs von Pflanzenschutzmitteln im Boden (1986)<br><br>SETAC guideline: Procedures for assessing the environmental-fate and ecotoxicity of pesticides: Part 1: Section 1.1: Aerobic degradation (1995)<br><br>OECD TG 307: Aerobic and anaerobic transformation in soil (2002) *<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 1.62 MBq/mg and radiochemical purity > 99%)<br><br>FOCUS Degradation Kinetics Report (2006, 2014)<br><br>GLP compliant | °C in:<br>Bruch West (loamy sand with a pH of 7.5): **35.47 d**<br>Li35b (loamy sand with a pH of 7.0): **8.98 d**<br>Lufa 2.2 (silty sand with a pH of 5.8): **9.20 d**<br>Mechenheim (silty sand with a pH of 6.8): **17.11 d** | 3.4-13% of AR of the substance remained in the extractable residues and 15.8-43.7% of AR in the non-extractable residues at day 120-121 of the study. Total recoveries were within 90.3-111.0 % of AR.<br><br>No significant amounts of radioactivity were detected in the volatile collection traps set up, however, a mineralization rates ($^{14}CO_2$) from 43.1 to 69.7% of AR was reported at the end of the study.<br><br>No degradates were identified. | dRAR B.8.1.1.1/02 |
| US EPA: Pesticide Assessment Guidelines Subdivision N: Series 162-1: Aerobic soil metabolism studies (1990)<br><br>BBA: Richtlinien für die Prüfung von Pflanzenschutzmitteln (Nr. IV, 4–1): Bestimmung des Verbleibs von Pflanzenschutzmitteln im Boden (1986)<br><br>SETAC guideline: Procedures for assessing the environmental-fate and ecotoxicity of pesticides: Part 1: Section 1.1: Aerobic degradation (1995)<br><br>OECD TG 307: Aerobic and anaerobic transformation in soil (2002) * | Non-normalized **best fit (FOMC) $DT_{50}$** value: **3.55 d** | The route and rate of degradation of mepiquat chloride in a loamy sand soil (Holly Springs, USA) with a pH of 5.7 was studied at 25 ± 1°C for 30 days.<br><br>The amount of residues (sum of combusted residues i.e. humin and remaining aqueous fraction i.e. fulvic and humic acids) was 15.6 %. Partitioning of the aqueous extract indicated that the majority of radioactivity was associated with the fulvic acid fraction.<br><br>Total radioactivity ranged from 88.5-108.5% of AR. Mepiquat chloride was reported to decline from 89.1% of AR at day 0 to 10.4% of AR on day 30; during this time analysis of gaseous traps found that $^{14}CO_2$ rose from 0.0% of AR on day 0 to 69.1% of AR on day 30.<br><br>The only degradate identified was 4-OH mepiquat-chloride which peaked at 1.6 % of AR in the day 7 | 1996<br><br>dRAR B.8.1.1.1/03 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| [2,6-$^{14}$C]-mepiquat chloride (specific activity 41.35 µCi/mg and radiochemical purity > 97%)<br><br>FOCUS Degradation Kinetics Report (2006, 2014)<br><br>GLP compliant | | sample. | |
| Non-guideline study<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 24.63 mCi/mMol and radiochemical purity unknown)<br><br>FOCUS Degradation Kinetics Report (2006, 2014)<br><br>Non GLP | Non-normalized best fit (**SFO) $DT_{50}$** value: **24.75 d** | The 60-day study was conducted using loamy sand with a pH of 6.8 at 20 ± 1°C.<br><br>In aerobic soil, the extractable residues declined from 73.3% of AR at day 0 (2 hours) to 15% of AR on day 60. Analysis indicated these extracts containing intact mepiquat chloride only whereas unidentified metabolites were present up to 6% of AR within the aqueous phase.<br><br>In sterile soil, levels of mepiquat chloride remained stable throughout the study varying from 67.8% of AR on day 0 to 68.3% of AR after 60 days including unextracted radioactivity reaching 27.5% of AR on day 60 (material balance ranged from 86.1-101.8% of AR throughout the study).<br><br>$CO_2$ or volatiles were not trapped and, thus, a complete material balance is not available.<br><br>As the FOMC kinetics did not improve the $\chi 2$ prosentual error and the visual fit, SFO kinetics is considered the most appropriately kinetics | 1979<br><br>dRAR B.8.1.1.1/04 |
| Photochemical degradation | | | |
| Photodegradation in water | | | |
| US EPA: Pesticide Assessment Guidelines Subdivision N: Series 161-2: Photodegradation studies in water (1982)<br><br>OECD TG 316: Photo transformation of Chemicals in Water – Direct Photolysis (2008) *<br><br>[2,6-$^{14}$C]-mepiquat | Mepiquat chloride was **stable** to aqueous photolysis under non-sensitised conditions and in the presence of a photosensitiser up to 24 d at 25 ± 1°C under light intensity of approximately 80 klx. | No mineralisation or formation of volatiles was detected in any of the irradiated or dark control samples over the course of the study. The material balance in irradiated samples ranged from a mean of 94.0 to 103.2% of AR. | 1990<br><br>dRAR B.8.3.1.2/02 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| chloride (specific activity 66.748 mBq/mmol and radiochemical purity > 98%)<br><br>GLP compliant | | | |
| Quantum yield | | | |
| BBA: Richtlinien für die Prüfung von Pflanzenschutzmitteln (Nr. IV, 6–1): Prüfung des Verflüchtigungsverhaltens und des Verbleibs von Pflanzenschutzmitteln in der Luft (1990)<br><br>Unlabelled mepiquat chloride (chemical purity 99.3%)<br><br>GLP compliant | The **absorption coefficients** were **0 L/mol/cm** for all wavelengths measured. | As the absorption coefficients of mepiquat chloride were zero for wavelengths from 295 to 800 nm, no quantum yield could be calculated. Hence, aqueous photolysis is unlikely to be a significant route of degradation of mepiquat chloride in environment. | 1991<br><br>dRAR B.8.3.1.2/01 |
| Photodegradation in soil | | | |
| US EPA: Pesticide Assessment Guidelines Subdivision N: Series 161-2: Photodegradation studies in soil (1982)<br><br>OECD guidelines for the testing of chemicals: proposal for a new guideline: Phototransformation of Chemicals on Soil Surfaces (2002) *<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 6.11 Ci/mole and chemical purity > 95%)<br><br>GLP compliant | There were **no significant differences** between the levels of mepiquat chloride remaining in dark and irradiated samples at at 25 ± 1°C. | A photolytic half-life could not therefore be determined due to no significant differences between the levels of mepiquat chloride remaining in dark and irradiated samples at the end of the 30 day study. | 1991<br><br>dRAR B.8.1.1.3/01 |
| Non-guideline study<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 24.63 mCi/mMoland chemical purity unkown)<br><br>Non GLP | Mepiquat chloride was found to be **photolytically stable** at 25°C. | A half-life could not be determined because mepiquat chloride was found to be photolytically stable.<br><br>The study was not conducted in accordance with any guideline or to GLP. However, the results are in line with the GLP study (B.8.1.1.3/01) and considered as supportive information. | 1979<br><br>dRAR B.8.1.1.3/02 |
| Photodegradation in air | | | |
| Atkinson method<br><br>non GLP | **Half-life ($t_{½}$)**: 4.56 h | The estimation of atmospheric half-life is based on the method of Atkinson. According the incremental method of Atkinson, the OH radical rate constant was estimated to be 28.14 x $10^{-12}$ $cm^3$ | 2001<br><br>dRAR B.8.5.1/01 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | | molecule$^{-1}s^{-1}$. The $DT_{50}$ value was based on a twelve hours day assuming an OH radical concentration of 1.5 x $10^6$ per $cm^3$. | |

* According to the dRAR, the study was conducted generally in line with the test method.

### 11.1.1 Ready biodegradability

A ready biodegradability study (**B.8.3.2.1/01**, **2003**) was available in the dRAR. The test followed OECD test guideline 301A "DOC Die-Away" test guideline (1992). Duplicate mixtures of test substance in concentration of 62.6 mg/L (lower concentration than the water solubility of mepiquat chloride, > 700 g/L) in a defined inorganic medium and a non-preadapted inoculum; activated sludge from laboratory wastewater plants treating municipal sewage (30 mg dw/l) were aerated at 22 ± 2°C. In addition, two blank controls, reference substance, inhibition control (inhibition of the inoculum by test substance), abiotic control and absorption onto the inoculum were tested in parallel. Degradation is followed by DOC analysis at frequent intervals over a 35-day period.

The degree of biodegradation is calculated by expressing the concentration of DOC removed (corrected for that in the blank inoculum control) as a percentage of the concentration initially present. The validity criteria were fulfilled and degradation of the reference substance, aniline, reached > 90% within the first 10 days. The lag-phase for the degradation was 19 days (before the 10% of degradation was reached). After that, the degradation accelerated resulting to 90-100% degradation within 10 days. Degradation of the inhibition control (containing mepiquat chloride and aniline) was 40-50% DOC after 14 days.

As the degradation (% of dissolved organic carbon removal) of the substance was higher than the trigger value of 70% within 28 days for the method, mepiquat chloride is considered readily biodegradable.

Ready biodegradability studies are among the preferred type of test data in the assessment of rapid degradability. The endpoint is presented in table (Table ) above.

### 11.1.2 $BOD_5$/COD

No studies available.

### 11.1.3 Hydrolysis

One study on hydrolytic degradation for mepiquat chloride was available in the dRAR. The study followed the US EPA guideline: Pesticide Assessment Guidelines: Subdivision N: Section 161-1: Hydrolysis studies (1982). The study conducted at 25°C and pH 3, 5, 7 and 9 in sterile aqueous buffer solutions (**B.8.3.1.1/01, 1989**) observed no degradation of mepiquat chloride over a 30-day period. Based on the results, the mepiquat chloride is considered **hydrolytically stable**.

Primary degradation studies i.e. via hydrolysis combined with hazard assessment of degradation products are among the preferred type of test data in the assessment of rapid degradability. The endpoints are presented in table (Table ) above.

### 11.1.4 Other convincing scientific evidence

#### 11.1.4.1 Inherent and enhanced ready biodegradability tests

No studies available.

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

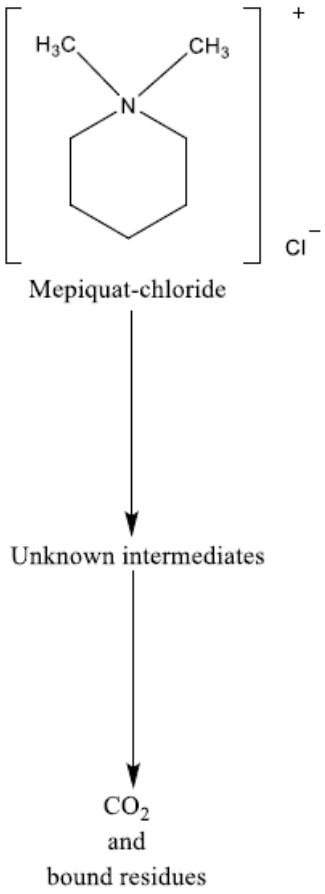
Aerobic mineralisation of mepiquat chloride in surface water was investigated under defined laboratory conditions in the dark (**B.8.3.2.2/01, 2016**) according the OECD test guideline 309 "Simulation biodegradation test" (2004). In conclusion, mepiquat chloride was found to be **stable**, or **degrading only very slowly** under the conditions of the test. Minor metabolites were observed with a maximum formation of 0.6% of AR and low amounts of $^{14}CO_2$ (maximum formation 4.8% of AR) and other volatiles (maximum formation 0.1% of AR) were reported. Surface water simulation studies are among the preferred types of test data in the assessment of rapid degradability.

One study on the route and rate of degradation of mepiquat chloride in water/sediment systems under aerobic conditions was reported in the dRAR. The study (**B.8.3.2.3/01, 2002**) basically followed the OECD test guideline 308 "Aerobic and anaerobic transformation in aquatic sediment systems" (2008). Mepiquat chloride was found to dissipate relatively rapidly from water, with the major route of dissipation being partitioning to sediment from the water phase; 37.3-47.5% of AR was present in the sediment matrix of both pond and stream systems within 7 DAT and peaking at 14-30 DAT (with 48.2-56.2% of AR). Following 30 DAT levels of radioactivity declined within the sediment to 26.7-27.6% of AR at 100 DAT. The decline in levels of radioactivity in the sediment was followed by an increase in mineralisation, in which levels of $^{14}CO_2$ rose from 4.7-13.2% of AR at 30 DAT to 61.7-65.8% of AR at 100 DAT. At 100 DAT, levels of radioactivity in the water phase had fallen from ~101% of AR (at 0 DAT) to < 1% of AR. The material balance of the study was 87.9-101.5% of AR throughout.

Further kinetic evaluation of the dissipation of mepiquat chloride was performed according to FOCUS Degradation Kinetics Report (2006, 2014). The whole system degradation rates of mepiquat chloride were calculated in accordance with current FOCUS Degradation Kinetics Report (**B.8.3.2.4/01, 2016**). SFO model had the best fit by most satisfactorily describing the decline for both water-sediment systems and the whole system $DT_{50}$ values for mepiquat chloride were calculated to be 32.01 and 32.58 days at 20°C (geometric mean 32.29 days). Based on the results, the **degradation** of mepiquat chloride is **not rapid** in natural environments.

Three studies of degradation in soil under aerobic conditions for mepiquat chloride were considered valid in the dRAR. Two of the studies (**B.8.1.1.1/02, 2003**) & **B.8.1.1.1/03, 1996**) were basically performed according the OECD test guideline 307 "Aerobic and anaerobic transformation in soil" (2002) and one (**B.8.1.1.1/04, 1979**) of them didn't follow any guidelines. The endpoints are presented in table (Table ) above. The studies were followed by further kinetic evaluation (**B.8.1.1.1/01, 2016**) according to FOCUS Degradation Kinetics Report (2006, 2014). The estimated half-lives of mepiquat chloride in soil ranged from 3.6 to 35.5 days. Based on the worst case scenario, mepiquat chloride **doesn't degrade rapidly** in soil under aerobic conditions. In addition to estimating the half-lives, the soil degradation studies were also assessed in order to address the degradation scheme for mepiquat chloride in soil. The proposed pathway scheme (Figure 1) is presented below.

**Figure 1. Proposed pathway scheme for the degradation of mepiquat chloride in soil.**




The endpoints are presented in table (Table ) above. However, since other data are available and water/sediment or soil fate studies are not among the preferred data to be used for assessing rapid degradability according to the CLP guidance, there is no need for further investigations of the data. These results do not impact the environmental classification but can be used as supportive information.

### 11.1.4.3 Photochemical degradation

One study (**B.8.3.1.2/02, 1990**) on photochemical degradation in water for mepiquat chloride conducted generally according to the OECD test guideline 316 "Photo transformation of Chemicals in Water – Direct Photolysis" (2008) wa available in the dRAR. The direct photolysis of mepiquat chloride was shown to be insignificant **as no photodegradation occurred** after several days of continuous exposure of 24 days at pH 7 at 25 ± 1°C. This is supported by measurements of the UV/visible absorption spectrum (**B.8.3.1.2/01, 1991**) according to BBA guideline Nr. IV, 6-1 (1990): the absorption coefficients of mepiquat chloride were zero for wavelengths of 295-800 nm, indicating aqueous photolysis not being a significant route of degradation of mepiquat chloride in the environment.

Also soil and air photolysis studies are available in the dRAR. The study (**B.8.1.1.3/01, 1991**) following the OECD test guideline draft "Photo transformation of Chemicals on Soil Surfaces" (2002) indicates the mepiquat chloride not degradating due to irradiation. The conclusion is supported by a non-guideline study (**B.8.1.1.3/02, 1979**) as well. However, mepiquat chloride entering the air (**B.8.5.1/01, 2003**) is subject to **rapid** indirect photochemical **degradation** ($DT_{50}$ value of 4.6 hours according the method of Atkinson).

The endpoints are presented in table (Table ) above. However, since other data (screening and simulation studies) is preceding over photolysis data for classification purposes, there is no need to investigate the data further. Therefore, detailed description of these field studies is excluded from this CLH report.

## 11.2 Environmental transformation of metals or inorganic metals compounds

**Not relevant for this proposal.**

## 11.3 Environmental fate and other relevant information

**A brief summary of studies considered reliable and relevant on environmental fate, listed in the Draft Renewal Assessment Report (dRAR), are reported below.**

**Table 10: Summary of relevant information on rapid environmental transformation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Environmental distribution | | | |
| Adsorption | | | |
| OECD TG 106: Adsorption/Desorption (1981)<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 0.111 mCi/mg and chemical purity 98.5%)<br><br>Non GLP | **Mepiquat chloride adsorption constants** in<br>**Soil type** / $K_f$* (mL/g) / $K_{oc}$ (mL/g) / **1/n**<br>**Sand** 0.22 191 0.720<br>**Loam** 9.88 1563 0.998<br>**Clay** 12.00 1099 0.998<br>**Sandy loam** 25.00 4833 0.999<br><br>*$K_f$ = Freundlich adsorption coefficient | The mobility of mepiquat chloride in soil based on $K_{OC}$ values can be considered as **slightly to moderately adsorptive**. | 1987<br><br>dRAR B.8.2.1/01 |
| OECD TG 106: Adsorption/Desorption (1981)<br><br>Unlabelled mepiquat chloride (chemical purity > 99%)<br><br>Non GLP | **Mepiquat chloride adsorption constants** in<br>**Soil type** / $K_f$* (mL/g) / $K_{oc}$ (mL/g) / **1/n**<br>**Clay loam** 1.71 67 0.97<br>**Light clay** 47.79 4685 1.027<br>**Sandy clay loam** 5.49 722 0.953<br>**Sand** 1.69 113 0.988 | The mobility of mepiquat chloride in soil based $K_{OC}$ values can be considered as **slightly to moderately adsorptive**. | 1991<br><br>dRAR B.8.2.1/02 |
| OECD TG 106: Adsorption -- Desorption Using a Batch Equilibrium Method (2000) *<br><br>[2,6-$^{14}$C]-mepiquat chloride (specific activity 164.37 µCi/mg and radiochemical purity unkown)<br><br>Non GLP | **Mepiquat chloride adsorption constants** in<br>**Soil type** / $K_f$* (mL/g) / $K_{oc}$ (mL/g) / **1/n**<br>**Pfungstadt (22°C)** 13.36 2304 0.972<br>**Pfungstadt (18°C)** 17.06 2942 0.980<br>**Neuhofen (22°C)** 5.74 216 0.963<br>**Neuhofen (18°C)** 7.41 278 0.933<br>**Lufa 2.1 (22°C)** 3.90 765 0.976<br>**Lufa 2.1 (18°C)** 5.17 1014 0.914<br><br>**Mepiquat chloride desorption constants (with $H_2O$)** in<br>**Soil type** / $K_f$* (mL/g) / $K_{oc}$ (mL/g) / **1/n**<br>**Pfungstadt (22°C)** 16.58 2859 0.991<br>**Pfungstadt (18°C)** 20.35 3509 0.906<br>**Neuhofen (22°C)** 7.79 293 0.948<br>**Neuhofen (18°C)** 10.12 380 0.878<br>**Lufa 2.1 (22°C)** 4.82 945 0.899<br>**Lufa 2.1 (18°C)** 9.05 1775 0.972 | The mobility of mepiquat chloride in soil based $K_{OC}$ values can be considered as **slightly to moderately adsorptive**. | 1978<br><br>dRAR B.8.2.1/03 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | **Mepiquat chloride desorption constants (with $H_2O$)** in<br>**Soil type** / **$K_f$*** (mL/g) / **$K_{oc}$** (mL/g) / **1/n**<br>**Pfungstadt (22°C)** 4.78 824 0.899<br>**Neuhofen (22°C)** 2.59 97 0.886<br>**Lufa 2.1 (22°C)** 1.48 290 0.767 | | |
| Volatilisation | | | |
| Laboratory volatilisation studies and theoretical estimations | | | |
| OECD TG 104: Vapour Pressure: Effusion method: isothermal thermogravimetry (2002)<br><br>[2,6-$^{14}$C]-mepiquat chloride (chemical purity 99.3%)<br><br>GLP compliant | **Vapour pressure:**<br><br>**< 1 x $10^{-8}$ Pa** at 20 °C and 25°C | Based on the low vapour pressure, **no significant volatilisation** is expected. | 2001<br><br>dRAR B.2.2/01 |
| Henry's law constant<br><br>not relevant<br><br>Non GLP | **2.994 x $10^{-12}$ Pa $m^3$ $mol^{-1}$** at 20 °C | Based on the calculated Henry's law constant being low, **no significant volatilisation** is expected. | 2004<br><br>dRAR B.2.2/02 |

* According to the dRAR, the study was conducted generally in line with the test method.

### 11.3.1 Summary of data/information on environmental fate and other relevant information

In the dRAR, three studies (**B.8.2.1/01, 1987; B.8.2.1/02, 1991 & B.8.2.1/03, 1978**) on adsorption in soils were considered valid for mepiquat chloride. Considering the measured $K_{OC}$ values ranging from 67 mL/g to 4685 mL/g, it is assumed that the substance is mobile to slightly mobile in the tested soils.

Based on the laboratory study (**B.2.2/01, 2001**) indicating very low vapour pressure (< 1 × $10^{-8}$ Pa, 20 °C) and and theoretical estimation (**B.2.2/02, 2002 & 2004**) of low Henry´s law constant (3.0 x $10^{-12}$ Pa $m^3$ $mol^{-1}$), mepiquat chloride is virtually non-volatile. Therefore, significant exposure to air is not to be expected.

The endpoints are presented in table (Table 10) above. However, these results do not impact the degradation classification and, therefore, no further investigations of the data is needed.

## 11.4 Bioaccumulation

A brief summary of studies considered reliable and relevant on bioaccumulation, listed in the Draft Renewal Assessment Report (dRAR), is reported below.

**Table 11: Summary of relevant information on bioaccumulation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Estimated bioaccumulation | | | |
| OECD TG 107: Partition Coefficient (n-octanol/water): Shake Flask | **N-octanol/water partition coefficient of mepiquat chloride at 20 °C** | Shake flask method is not applicable to surface active substances. | 2000<br><br>dRAR B.2.7/01<br><br>**Key study** |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Method (1995)<br><br>[2,6-$^{14}$C]-mepiquat chloride (chemical purity 99.3 %)<br><br>GLP compliant | log $P_{ow}$ = **-3.45** (deionised water)<br>log $P_{ow}$ = **-3.20** (pH 4)<br>log $P_{ow}$ = **-3.55** (pH 7)<br>log $P_{ow}$ = **-3.14** (pH 10) | However, the values observed in the test are in line with expectations for a salt solution. | |

### 11.4.1 Estimated bioaccumulation

No studies available.

### 11.4.2 Measured partition coefficient and bioaccumulation test data

No bioaccumulation studies were included in the dRAR. In the absence of experimental results on BCF values, the bioaccumulation for classification purposes can be based on substances physico-chemical properties such as partition coefficients. The partition coefficient for mepiquat chloride was estimated by conducting tests (**B.2.7/01, 2000**) according to OECD test guideline 107 "Partition Coefficient (n-octanol/water): Shake Flask Method" (1995). The study considered valid on partition coefficient n-octanol/water resulted in log $P_{OW}$ values from -3.14 to -3.55. Thus, despite the fact that octanol cannot be used as a surrogate of lipid sorption for a surface-active substance, there is no indication of the substance having a a high bioaccumulation potential.

The endpoints are presented in table (Table 11) above.

## 11.5 Acute aquatic hazard

Mepiquat chloride is a hygroscopic substance and after manufacture dry technical material is diluted with water to form a stable "technical concentrate" containing 615-665 g/L mepiquat chloride (codenamed BAS 083 52 W). Some of the ecotoxicological studies were conducted with this technical concentration or with the concentration even lower (51.6 g/L). However, in the studies the test concentrations expressed as mg a.s./L refer to the concentrations of mepiquat chloride and results are based on the active substance content. This applies also for chronic studies. All available data on acute aquatic toxicity are summarized in the following table. The new studies submitted to support PPP renewal process of mepiquat chloride are acute studies on sheepshead minnow (*Cyprinodon variegatus*), saltwater mysid (*Mysidopsis bahia*), eastern oyster (*Crassostrea virginica*), duckweed (*Lemna gibba*) and green algae (*Pseudokirchneriella subcapitata*).

**Table 12: Summary of relevant information on acute aquatic toxicity**

| Method | Species | Test material | Results[1] | Remarks | Reference |
|---|---|---|---|---|---|
| **Fish** | | | | | |
| 96 hrs<br>static<br>EPA 72-1, EPA-SEP 540/9-85-006, OECD 203<br>GLP | Rainbow trout *(Oncorhynchus mykiss)* | Mepiquat chloride (powder)<br><br>purity 99.0 % | $LC_{50}$ > 100 mg a.s./L (nom) | Two test concentrations 50 and 100 mg a.s./L. Measured concentrations were between 93.3 to 100.4 % and between 86.7 to 100.7 % of nominal at test initiation and termination, respectively. No undissolved test substance was visible. There was no mepiquat chloride related | dRAR B.9.2.1. CA 8.2.1/1 (1991b) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | mortality. Two control fish died at 96 h, exceeding validity criterion (> 10 % in controls). This was not considered critical as no mortalities in any other group were observed. | |
| 96 hrs static OECD 203, EPA 72-1, EPA-SEP 540/9-85-006 GLP | Bluegill sunfish (*Lepomis macrochirus*) | Mepiquat chloride (powder) purity 99.0 % | $LC_{50}$ > 100 mg a.s./L (nom) | Two test concentrations 50 and 100 mg a.s./L. Test substance was satisfactorily maintained in the test solution 85.1 – 100.6 % from nominal, except in one replicate (78.1 % at 96 h). No undissolved test substance was visible. The OECD 203 validity criteria were fulfilled. There was no mepiquat chloride related mortality. | dRAR B.9.2.1.1. CA 8.2.1/2 (1991a) |
| 96 hrs static EPA 72-3 (a) GLP | Sheepshead minnow *(Cyprinodon variegatus)* | Mepiquat chloride (clear liquid) purity 54.6 % | $EC_{50}$ > 151 mg a.s./L (mm) | Five test concentrations tested. All test solutions were clear and colorless, indicating that the test substance was soluble at all levels tested. The OECD 203 validity criteria were met (mortality in the control below 10 %, DO > 60 % in the static test condition and the recovery of test concentrations > 80 % of nominal throughout the study). The test substance was satisfactorily maintained in the test solution (110 – 126 %). | dRAR B.9.2.1.1. CA 8.2.1/3 (1995c) |
| **Aquatic invertebrates** | | | | | |
| 48 hr static EEC 79/831 A V C 2 OECD 202 GLP | *Daphnia magna* | Mepiquat chloride (greyish white solid) purity 99 % | **$EC_{50}$ = 68.5 mg a.s. / L (nom)** | Seven test concentrations tested. Measured concentrations ranged between 93.4-102.3 % and 93.9-103.1 % of nominal at initiation and termination of the test, respectively. No significant deviation from the guideline. Validity criteria OECD 202 fulfilled. | dRAR B.9.2.2.1. CA 8.2.4.1 (1991a) |
| 48 hr static EPA 72-2(a) GLP | *Daphnia magna* | Mepiquat chloride (clear liquid) purity 54.6 % | $EC_{50}$ = 106 mg a.s./l (mm) | Five test concentrations tested. All test solutions were clear and colorless after mixing. Validity criteria of OECD 202 fulfilled. No significant deviation from the guideline. Test substance was maintained 90.0-109.7 % of nominal concentrations. | dRAR B.9.2.2.1. CA 8.2.4.1 (1994a) |
| 96 hr static EPA 72-3(b) GLP | *Mysidopsis bahia* | Mepiquat chloride (clear liquid) purity 54.6 % | $EC_{50}$ = > 136 mg a.s./l (mm) | Five test concentrations tested. All test solutions appeared clear and colorless, indicating that the test substance was soluble at all levels tested. Two replicate test chambers with 10 mysids in each. No significant deviations from the US EPA OCSPP 850.1035 test guideline. Mean measured concentrations were 19, 29, 49, 79 and 136 mg a.s./L. | dRAR B.9.2.2.2. CA 8.2.4.1/2 (1995b) |

| | | | | | |
|---|---|---|---|---|---|
| 96 hr<br>flow-through<br>EPA 72-3 (c)<br>GLP | Eastern Oyster<br>*(Crassostrea virginica)* | Mepiquat chloride (clear liquid)<br><br>purity 54.6 % | **$EC_{50}$ = 15 mg a.s./L (mm)** | Study was performed according to US EPA 72-3(c) (equivalent to OCSPP 850.1025). For each concentrations there was only one replicate containing 20 organisms, No other significant deviation from US EPA OCSPP 850.1025 test guideline were apparent. All test solutions were clear and colorless, indicating that the test substance was soluble at all levels tested. Mean measured concentrations ranged from 66 % to 112 % of nominals. | dRAR B.9.2.2.2. CA 8.2.4.2/2 (1995a) |
| **Algae** | | | | | |
| 72 hrs<br>static<br>OECD 201<br>GLP | *Pseudokirchneriella subcapitata* | Mepiquat chloride<br><br>purity 99 % | $E_rC_{50}$ = >1000 mg a.s./L (nom) | Six test concentrations. 5 replicates per treatment group and 10 in the control. Homogeneity of the test substance was proved by analysis. Measured concentrations were between 103.3 and 106.9 % of nominal (tested concentrations were the lowest, mid and highest concentration). Validity criteria of OECD 201 were partially fulfilled: Growth rate in controls fulfilled the criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate control during the whole test fulfilled the criterion < 7 %. The mean coefficient of variation for section-by section specific growth rates exceeded the criterion (41.5% > 35%). | dRAR B.9.2.3.1. CA 8.2.6.1 (1993a) |
| 96 h<br>static<br>ASTM E 1218-90<br>OECD 201<br>EPA 850.1000<br>GLP | *Anabaena flos-aquae* | BAS 083 52 W (617.6 g/L Mepiquat chloride, water based liquid) (nominal 600 g mepiquat chloride/L) | **72 h $E_rC_{50}$ = 48.2 mg a.s./L (nom.)** | Five test concentrations plus control. Five replicates per treatment group and 10 replicates for the control. Measured concentrations were 90-109.0 % of nominals (all the concentrations were tested). Validity criteria of OECD 201 were partially fulfilled: Growth rate in controls fulfilled the criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate control during the whole test fulfilled the criterion < 7 % (2.8%). The mean coefficient of variation for section-by section specific growth rates (days 0-2, 2-3, 3-4) exceeded the criterion (64.3% > | dRAR B.9.2.3.2. CA 8.2.6.2 (2002a) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 35%). However over days 0-2 and 2-3 it was 32.9% meeting requirement of ≤ 35 %. Test is considered valid up to 72 h. | |
| 72 hrs static OECD 201 GLP | *Pseudokirchneriella subcapitata* | Mepiquat chloride<br><br>purity 98.1 % | $E_bC_{50}$ = >1000 mg a.s./L (nom)<br>$E_rC_{50}$ = >1000 mg a.s./L (nom) | Five test concentrations plus control. Three replicates per treatment group and six in the control. Measured concentrations were between 93.5 and 104.0 % of nominal (all the concentrations were tested).<br><br>The validity criteria of OECD 201 are fulfilled: Increase in cell density was 195.4-fold within 72 hours. Growth rate in controls was 1.763 ± 0.019 1/d fulfilling the the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 1.3 % fulfilling the validity criterion < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) was 7.2 % fulfilling the validity criterion < 35 %). | dRAR B.9.2.3.1 CA 8.2.6.1 (2019) |
| **Aquatic plants** | | | | | |
| 7 day static OECD draft guideline (Oct.2000 ) Lemna sp. growth inhibition test EPA 850.4400 ASTM E 1415-91 GLP | *Lemna gibba* | BAS 083 52 W (617.6 g/L Mepiquat chloride, water based liquid formulation, nominal 600 g/L) | **$E_rC_{50}$ = 17.45 mg a.s./L** (based on geometric mean measured concentrations) | Six test concentrations plus control. 3 replicates per treatment group and 6 for the control. Concentrations were measured from three highest concentration and were 86.4-105.4 % of nominals at the initation of the test and 31.6-117.7 % at the termination. Test concentrations we unstable at the lowest measured concentrations (1.0 mg a.s./L). Statistically significant inhibition was already observed from 0.10 mg a.s./L (nominal). $EC_{50}$ value falls within the dose range where reliable analytical data is available. Doubling time of frond number in the control was 2.1 fulfilling the OECD 221 criteria (< 2.5 d). Only frond number was measured. | dRAR B.9.2.4. CA 8.2.7/1 (2003) |
| 7 day static OECD 221 | *Lemna gibba* | BAS 083 34 W (51.6 g/l mepiquat chloride, pinkish liquid, nominal 50.0 g/L) | **$E_rC_{50}$ = 31.77 mg a.s. /L (meas.)** | Doubling time in controls 1.6 days, validity criterion of OECD 221 was met (<2.5 d). Test medium was clear throughout the test. Both frond numbers and dry weight were assessed, frond number being most sensitive endpoint. Results are based on | dRAR B.9.2.4. CA 8.2.7/2 (2017) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | geometric mean measured concentrations. | |

mm = mean measured; nom = nominal
Test concentrations expressed as mg a.s./L refer to the concentrations of mepiquat chloride and results are based on the active substance content.

### 11.5.1 Acute (short-term) toxicity to fish

Acute toxicity data with mepiquat chloride was available on three fish species; rainbow trout, bluegill sunfish and sheepshead minnow (see Table 12 above).

All studies were deemed reliable in the draft version of Renewal Assessment Report (2018) of mepiquat chloride. There were no mepiquat chloride related toxicity in either of these studies and 96 hr-$LC_{50}$ values were > 100 mg a.s./L, > 100 mg a.s./L. and 151 mg a.s./L, respectively. Results were based on nominal concentrations as the test substance was satisfactorily maintained in the test solutions in all studies. The $LC_{50}$ and NOEC values were estimated by visual inspections of mortality and observation data. Studies were conducted according to OECD guideline 203 following GLP and no significant deviations from the test guideline were identified. In the study with rainbow trout two control fish died at 96 hours, exceeding the OECD 203 validity criterion (mortality < 10 % in controls). However, as there were no mortalities in any other group this was not considered critical. Based on the available studies mepiquat chloride is not acutely toxic to aquatic fish up to the maximum concentration tested.

### 11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Two acute studies with *Daphnia magna* was available and one with saltwater mysid *Mysidopsis bahia* and one with eastern oyster *Crassotrea virginica* (see Table 12 above).

***Daphnia*** studies (48 h static tests) were conducted according to test guidelines OECD 202 and EPA 72-2(a) (performance of the test is similar to the OECD 202), and no significant deviations from the guidelines were apparent. The validity criteria of OECD 202 were fulfilled (dissolved oxygen > 3 mg/L, no immobilisation in controls). Test substance was sufficiently maintained in both studies (93.5-102.3 % and 90.0 – 109.7%) and dose responses were obtained; an $EC_{50}$ values of 68.5 mg a.s./L and 106 mg a.s./L were determined.

The acute toxicity of mepiquat chloride to ***Mysidopsis bahia*** was determined in a 96-hour static test which was performed according to US EPA 72-3(b) test guideline (equivalent US EPA OCSPP 850.1035) and following GLP. No significant deviations from the US EPA OCSPP 850.1035 guideline were apparent. The test solutions were sufficiently maintained in the test solutions (mean measured were 19, 29, 49, 79 and 136 mg a.s./L). After 96 hours of exposure, mortality in the highest concentration 136 mg a.s./L was 10%. In the negative control and in all other concetrations no signs of toxicity were observed. $LC_{50}$ values at 24, 48, 72 and 96 hours were all > 136 mg a.s./L.

In the test with ***Crassotrea virginica*** the acute toxicity of mepiquat chloride on the shell deposition during 96-h esposure period was determined under flow-through condition. The oysters were exposed to nominal concentrations of 3.9, 6.5, 11, 18, 30 and 50 mg a.i./L (mean measured 4.0, 7.3, 12, 18, 30 and 33 mg a.s./L) for 96 hours. The study was peformed according to US EPA 72-38(c) test guideline (equivalent to OCSPP 850.1025) and following GLP. For each concentration (six test concentrations and one negative control) there were only one replicate containing 20 organisms. Due to a miscount at test initiation, the control test chamber contained 18 oysters. No other significant deviations from the US EPA OCSPP 850.1025 test guideline were apparent. Temperature was 29.0 – 30.5 °C during the study and pH 8.0-8.2. No mortalities occurred among oysters in the control or treatment groups. The shell growth was significantly reduced in the 18, 30 and 33 mg a.s./L treatments in comparison to the control. Mean measured concentrations ranged from 66 to 112% of nominal concentrations and they were used in the estimation of $EC_{50}$ value which was determined to be 15 mg a.s./L.

Based on the available studies mepiquat chloride is not considered acutely toxic for aquatic invertebrates. However, the lowest effect value for acute toxicity was observed for eastern oyster ($EC_{50}$ 15 mg a.i./L).

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

Three algae studies and two studies with aquatic plant *Lemna gibba* were available (see Table 12 above).

*Studies with algae*

*Study 1*

The effect of mepiquat chloride (purity 99%) on the growth of green alga ***Pseudokirchneriella subcapitata*** was determined over a 72-hour exposure period. Mepiquat chloride was tested at nominal test concentrations of 10, 25, 75, 150, 400 and 1000 mg/L with a control tested in parallel. There were 5 replicates per treatment group and 10 replicates for the control. Initial cell density was 4 x $10^4$ cells/mL. Cell concentrations in each flask was determined 24, 48 and 72 hours after the start of the experiment using photometer. Concentrations of the test item were confirmed by analysis of treated growth medium at the start and after 72-hours (concentrations tested were 10, 150 and 1000 mg a.s./L) and found to be between 103.3 and 106.0% of nominal. Temperature during the test was 22-24 °C and pH varied 7.89-8.38.

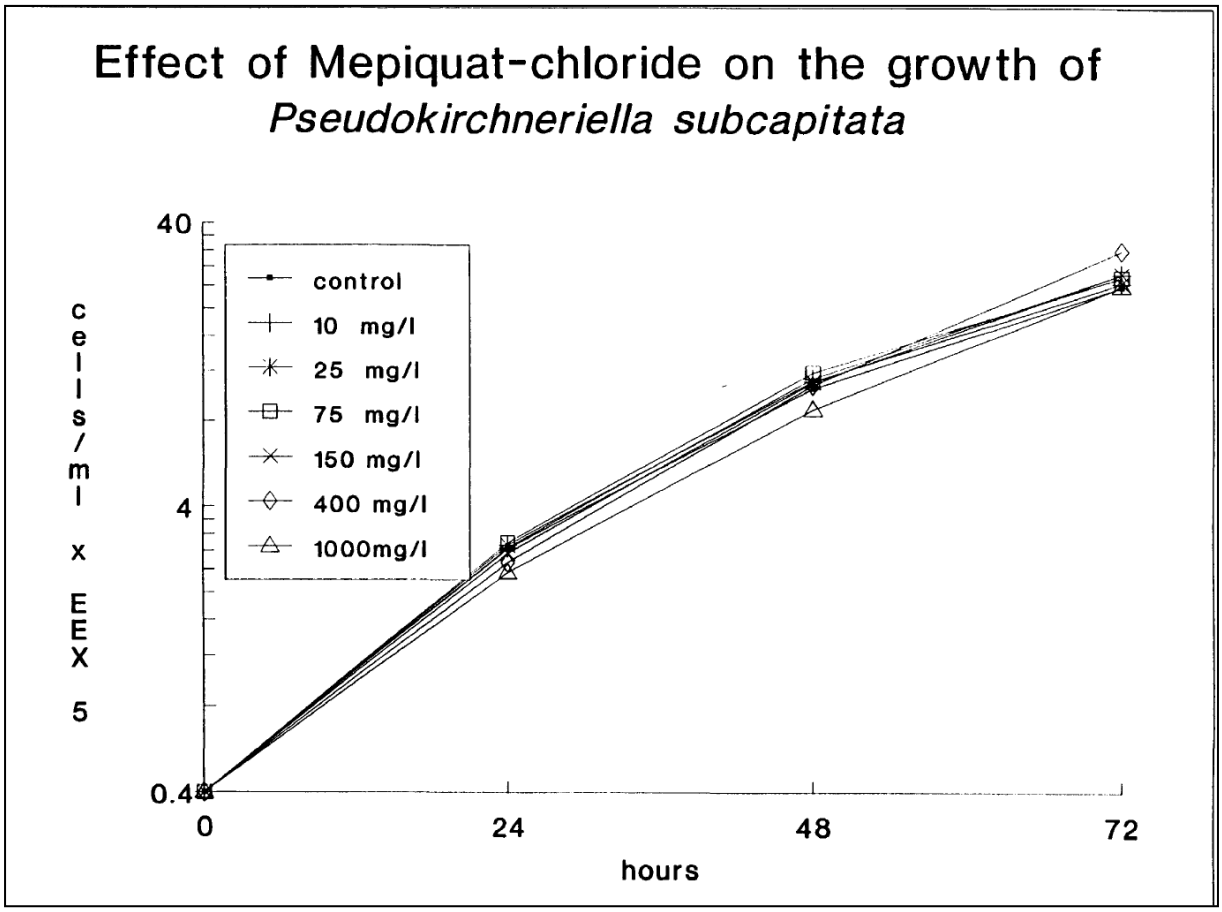
Growth inhibition in the form of biomass and growth rate was assessed after 72 hours. Mepiquat chloride had no negative effect on algal biomass up to the highest concentration tested (1000 mg/L), although at this concentration biomass reduced. At all other concentrations, biomass was seen to increase with a maximum biomass observed at 400 mg/L, an increase of 16.8% compared with controls, that was significant. There was no inhibition of algal growth rate as a result of exposure to mepiquat chloride. Morphological effects on algae could not be observed.

Based on nominal concentrations, the $E_bC_{50}$ (biomass) and $E_rC_{50}$ (growth rate) were both > 1000 mg a.s./L.

The study was conducted according to OECD 201 guideline following GLP. The purity of the batch used in this test (99 %) is within the current specification of dry mepiquat chloride. Test substance consentrations were maintained sufficiently during the test (measured concentrations 103.3 - 106% of nominal). The validity criteria of OECD 201 were only partially fulfilled: Growth rate in controls was 1.360 ± 0.015 1/d fulfilling the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 1.1 % fulfilling the validity criterion < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) was, however, 41.6 % exceeding the criterion (< 35 %). The average growth rate during 0 – 1, 1- 2 and 2 – 3 days was 2.0, 1.3 and 0.8, respectively. A similar reduction in growth rates is observed for the treatments (see figure below).

During the Peer Review Process of mepiquat chloride it was noted that “During the 48–72 hour period, growth in all concentrations dropped below the overall mean growth rate and growth rates exceeded that in the controls at the two highest concentrations tested, suggesting that there were very limited effects. The section-by-section growth rate criteria were introduced to ensure that variation within the controls does not undermine the ability to detect toxic effects. It would also identify whether there was a defined lag in growth that could indicate a recovery from a toxic effect with time; neither of the above were evident in this study. Considering that there were very clearly no toxic effects on this organism, the minor exceedance in the section-by-section growth rate of the controls does not impact on the validity of the results.” This argument was considered relevant although the validity criteria of OECD 201 were strictly speaking not met. Considering the growth curves (see Figure 2 below) and that no significant inhibition was observed, the minor deviation from the validity criterion can be accepted in this case. Dossier submitter is also in favour of accepting this study for classification purpose.

**Figure 2. The effect of mepiquat chloride on the growth of *Pseudokirchneriella subcapitata***




*Study 2*

The other algae study was conducted with blue-green alga ***Anabaena flos-aquae***. The effect of mepiquat chloride (purity 617.6 g mepiquat chloride/L, water based liquid) was determined over a 96-hour exposure period. Mepiquat chloride was tested at nominal test concentrations of 1, 3, 10, 30 and 100 mg/L with a control tested in parallel. There were 5 replicates per treatment group and 10 replicates for the control. Initial cell density was 3 x $10^4$ cell/L.

Cell concentration in each flask was determined 48, 72 and 96 hours after the start of the experiment, using a spectrophotometer. Test concentrations were confirmed by analysis from all concentrations and found to be between 90.0 and 109.0% of nominal at the start of the trial and 93.7-107.8 % after 96 hours. Measured concentrations are presented in the Table 13 below. Temperature varied 21-23 °C and pH 7.38-7.46.

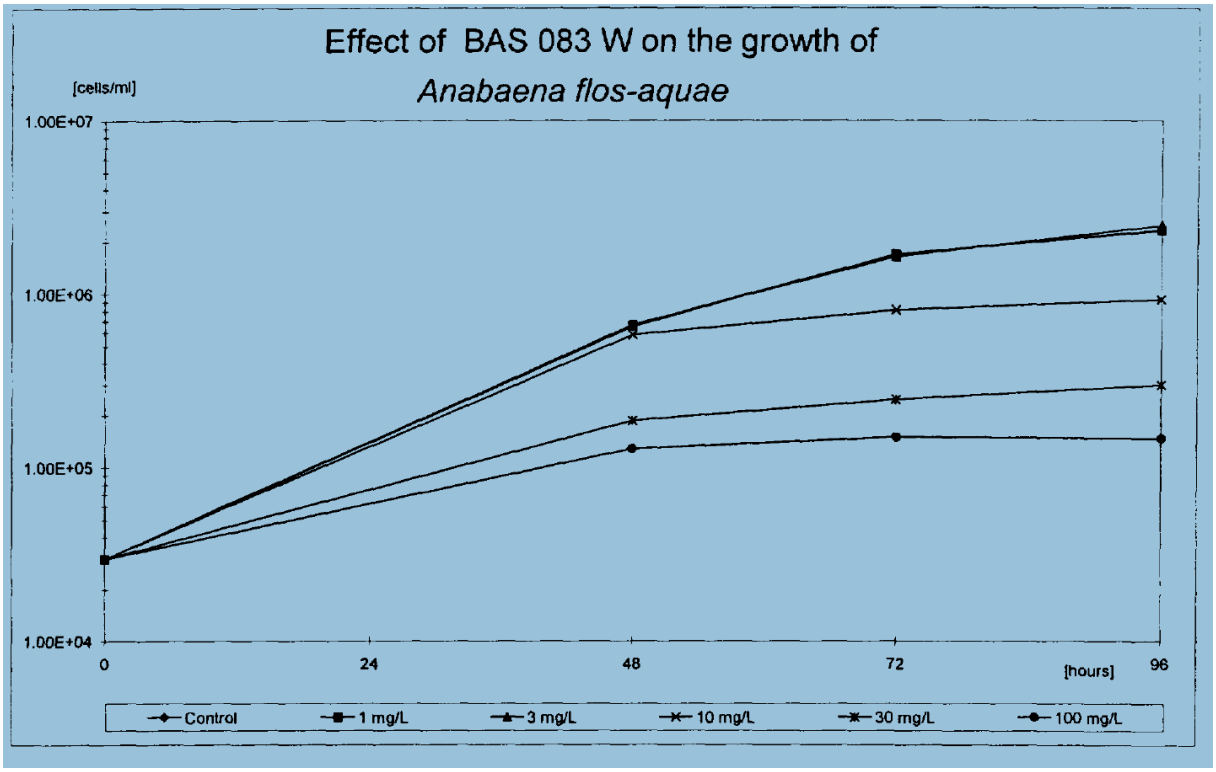
**Table 139: Measured concentrations of mepiquat chloride in the exposure solutions**

| Nominal concentration (mg a.s./L) | Mean Measured concentration (mg a.s./L) | | | |
|---|---|---|---|---|
| | 0-Hour | % Nominal | 96-Hour | % Nominal |
| 1 | 0.90 | 90.0 | 0.937 | 93.7 |
| 3 | 2.88 | 96.0 | 2.99 | 99.7 |
| 10 | 9.57 | 95.7 | 10.58 | 105.8 |
| 30 | 31.00 | 103.3 | 32.31 | 107.7 |
| 100 | 108.99 | 109.0 | 107.82 | 107.8 |

Growth inhibition in the form of biomass and growth rate was assessed after 96 hours. Based on nominal concentrations, the $E_bC_{50}$ (biomass) was 14.4 mg a.s./L (95% confidence interval 13.7 – 15.2) and $E_rC_{50}$ (growth rate) was 44.8 mg a.s./L (95% confidence interval 41.5 – 48.3).

The study was conducted according to OECD 201 guideline following GLP. The validity criteria of OECD 201 were partially fulfilled: Growth rate in controls was 1.089 ± 0.016 1/d fulfilling the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 2.8 % fulfilling the validity criterion of < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-2, 2 - 3 and 3 - 4) was, however, 64.3 % exceeding the criterion (< 35 %). The average growth rate during 0 – 2, 2 - 3 and 3 - 4 days was 1.5, 1.0 and 0.3, respectively. A similar reduction in growth rates is observed for the treatments (see Figure 3 below).

**Figure 3. The effect of BAS 083 W on the growth of *Anabaena flos-aquae***




During the Peer Review Process it was noted that the mean coefficient of variation for the section-by-section growth rate in the controls over days 0-2 and 2-3 was 32.9 % which meets the requirement of ≤ 35%. Considering that a algae study is normally performed up to 72 hours, the study was considered valid up to 3 days (72 hours). The endpoints calculated for 72 hours ($E_rC_{50}$ = 48.241 mg a.s./L) were provided and are presented in the Table 140 below. Dossier submitter is in favour of considering this study acceptable up to 72 h also for classification purpose.

**Table 140: The effect of BAS 083 W on the growth of *Anabaena flos-aquae* - 72-hours endpoints**

| Test species | Test material | Test System | Endpoint (95% confidence limits) |
|---|---|---|---|
| *Anabaena flos-aquae* | BAS 083 W | 72 - hour | **$E_rC_{50}$ = 48.241** (45.574 – 51.176)<br>$E_rC_{20}$ = 10.289 (9.441 – 11.139)<br>$E_rC_{10}$ = 4.588 (4.052 – 5.137)<br><br>$E_bC_{50}$ = 10.048 (9.773 – 10.328)<br>$E_bC_{20}$ = 4.928 (4.699 – 5.150)<br>$E_bC_{10}$ = 3.396 (3.186 – 3.601) |

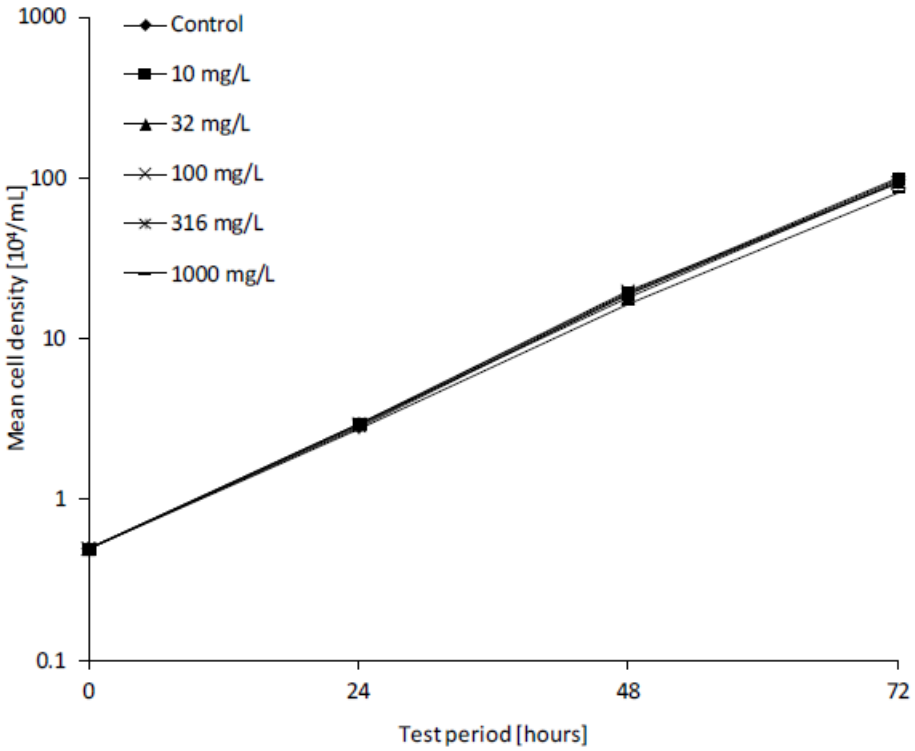
*Study 3*

In the third study, the effects of mepiquat-chloride (purity 98.1%) on the growth of the freshwater green algae, ***Pseudokirchneriella subcapitata***. Algae were exposed to three replicates of five test concentrations (10, 32, 100, 316 and 1000 mg a.s./L) and six replicates for the control. Initial cell density was 5000 cells/mL. Cell density in each test vessel was determined at 24, 48 and 72 hours after the start of the test by spectrophotometric measurements. All the concentrations of the test item were confirmed by LC-MS/MS of test medium at the start (0-hours) and test termination (72-hours) found to be between 93.5 and 104.0% of nominal. Temperature during the test was 22.0-22.5 °C and pH varied 7.9-9.6.

Growth inhibition in the form of biomass and growth rate was assessed after 72 hours. Based on nominal concentrations, the $E_bC_{50}$ (biomass) and $E_rC_{50}$ (growth rate) were both > 1000 mg a.s./L. Morphological effects on algae were assessed but not observed.

**The validity criteria of OECD 201 are fulfilled: increase in cell density was 195.4-fold within 72 hours. Growth rate in controls was 1.763 ± 0.019 1/d fulfilling the the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 1.3 % fulfilling the validity criterion < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) was 7.2 % fulfilling the validity criterion < 35 %). A similar trend was observed in the test concentrations as well (see Figure 4 below).**

**Figure 4. The effect of mepiquat chloride on the growth of *Pseudokirchneriella subcapitata***




*Studies with other aquatic plants – Lemna gibba*

*Study 1*

The effect of BAS 083 52 W (mepiquat chloride 617.6 g/L, water based liquid formulation) on the growth of the duckweed ***Lemna gibba*** was determined over a 7-day exposure period. The study was performed according to the draft OECD 221 test guideline following GLP. There were 3 replicates per treatment

group and 6 replicates for the control containing 3 fronds from 4 plants. Test vessels were inoculated with less than 10-day old Lemna. Vessels were covered and placed on trays and incubated under controlled environment conditions for 7 days under continuous light.

A stock solution was prepared by dissolving 167 g of BAS 083 52 W (equivalent to 100 mg mepiquat chloride) in 1000 g of growth medium and stirred constantly. From this stock solution, further dilution were made to give nominal concentrations of 0.001, 0.01, 0.10, 1.0 10.0, and 100 mg a.s./L. Controls were exposed to untreated growth medium.

Frond production and appearance were recorder on days 3, 5 and 7, the number of fronds visibly projecting from the parent frond was counted.

Growth inhibition in the form of frond number and growth rate was assessed after 7 days. Concentrations were confirmed by chemical analysis of treated medium at the start of the test and after 7-days. Concentration analyses were taken from three highest test concentrations 1, 10.0 and 100 mg a.s./L as these were above the LOQ. Analyses confirmed test concentrations were in a range of 86.4 – 105.4% of nominal at the start of the test, and between 31.6 – 117.7% of nominal at the end (see Table 15 below). Temperature during the study was 24-26 °C and ph 8.42-8.51.

**Table 15: Measured concentrations of mepiquat chloride in the three highest exposure solutions**

| Nominal concentration (mg a.s./L) | Mean measured concentration (mg a.s./L) | | | | Geometric mean measured (mg a.s/L) |
|---|---|---|---|---|---|
| | 0-Day | % Nominal | 7-Day | % Nominal | |
| 1 | 1.0 | 99.8 | 0.32 | 31.6 | 0.57 |
| 1 | 0.97 | 97.4 | 0.36 | 35.7 | |
| 10 | 10.24 | 102.4 | 10.24 | 102.4 | 10.25 |
| 10 | 10.32 | 103.2 | 10.15 | 101.5 | |
| 100 | 86.36 | 86.4 | 97.02 | 97.0 | 100.94 |
| 100 | 105.39 | 105.4 | 117.67 | 117.7 | |

Growth in control cells was good, with fronds increasing from 12 to 121 per vessel corresponding to a doubling time of 2.1 days (10.1 multiplication) fulfilling requirement in the OECD 221 (doubling time of less than 2.5 days). Statistically significant inhibition was observed at nominal concentration from 0.10 mg a.s./L. At a concentrations of 10 mg a.s./L, a reduced ability in separating frond was reported. At 100 mg/L, only single fronds were observed (no colonies) (see Table 16 below).

**Table 16: Percentage growth inhibition of *Lemna gibba* after 7 days exposure to mepiquat chloride**

| Nominal concentration (mg a.s./L) | % Inhibition in 7 days | |
|---|---|---|
| | Growth rate | Frond number |
| 0.001 | 0.76 | 0.30 |
| 0.01 | 5.66 | 10.98 |
| 0.10 | 12.75* | 28.35* |
| 1.0 | 18.50* | 38.72* |
| 10.0 | 32.11* | 58.23* |
| 100 | 83.36* | 94.82* |
| **Endpoints (mg a.s./L)** | | |
| $E_rC_{50}$ (7-day) | 15.41 (13.53 – 17.56[1]) | |
| $E_rC_{10}$ (7-day) | 0.15 (0.12 – 0.2[1]) | |
| $E_bC_{50}$ (7-day) | 2.6 (2.28 – 2.96[1]) | |
| $E_bC_{10}$ (7-day) | 0.01 (0.01 – 0.02[1]) | |
| NOEC | 0.01 | |
| LOEC | 0.01 | |

* Statistically significant ($\alpha = 0.05$)
[1] 95% confidence interval

In the test report the results were based on nominal concentrations, the 7-day $E_bC_{50}$ (biomass) was 2.6 mg a.s./L (95% confidence interval 2.28 – 2.96) and $E_rC_{50}$ (growth rate) was 15.41 mg a.s./L (95% confidence interval 13.53 – 17.56). Because unstable exposure conditions were observed at low mepiquat concentration (1.0 mg a.s./L), the results were also calculated based on geometric means of the concentrations in the three highest exposure concentrations (nominal 1, 10 and 100 mg/l; measured geom. mean: 0.57; 10.25 and 100.94 mg/l) resulting; **$EC_{50}$ value of 17.45 mg a.s./L**.

Another deviation (in addition to that the results were initially based on nominal concentrations although at 1 mg/l the measured concentrations at the end of the study were only 31.6 – 35.6 % of nominal) from the OECD 221 test guideline was that only frond number was measured. As according to the test guideline at least one other measurement variable (total frond area, dry weight or fresh weight) should also be measured since some substances may affect other measurement variables much more than frond numbers. There is another Lemna study available (Study 2) where both frond number and dry weight were measured and the frond number was the most sensitive endpoint. Therefore, it might be considered that the lack of another variable is not devalidating this study, keeping in mind that the test substances are not completely the same in these studies.

As a conclusion dossier submitter is proposing to use this study and obtained $E_rC_{50}$ value of 17.45 mg a.s./L (based on geometric mean measured concentrations) for classification purpose as this value falls within the dose range where reliable analytical data is available.

*Study 2*

The effect of BAS 083 34 W (mepiquat chloride 51.6 g/L, pinkish liquid) on the growth of the duckweed *Lemna gibba* was determined in a static test over a 7-day exposure period. Test was conducted according to OECD 221 and EC No 761/2009, C26 (2009) guidelines and in compliance with GLP. Test item BAS 083 34 W is noted to contain only one co-formulant in very low concentration which do not have harmonised or self-classification as hazardous to the aquatic environment. The rest of the test item is water. The detailed content is provided in the confidential annex. The results are based on the active substance mepiquat chloride content.

In the test duckweed was exposed to seven cocentrations of BAS 083 34 W with a control run in parallel. There were 3 replicates per test group containing 12 fronds (4 fronds from 3 plants). Test vessels were

covered and incubated under controlled environmental conditions under continuous light. A stock solution of 1000 mg test item/L was prepared by dissolving 1031 mg of test item into 1031 mL of growth medium by intense stirring for 15 minutes. From this stock solution, further dilutions were made to give nominal concentrations of 1.0, 3.2, 10, 32, 100, 316, and 1000 mg test item/L. Controls were exposed to untreated growth medium. Concentrations were measured from treated medium at the start of the test and after 7-day. Analyses confirmed test item concentrations were in a range of 105 – 115% of nominal at the start of the test, and < LOQ (limit of quantification of 0.026 mg a.s./L) – 84% at the end (see Table 17 and Table 18 below).

**Table 17: Summary of Analytical Results**

| Nominal concentration (mg test item/L) | % Nominal[1] | | | |
|---|---|---|---|---|
| | 0-Day | % RSD | 7-Day | % RSD |
| Control | < LOD | n.a | < LOD | n.a |
| 1.0 | 115 | 1 | < LOQ | n.a |
| 3.2 | 113 | 3 | 9* | n.a |
| 10 | 114 | 0 | 12* | n.a |
| 32 | 112 | 0 | 36 | 1 |
| 100 | 105 | 1 | 67 | 1 |
| 316 | 110 | 0 | 80 | 7 |
| 1000 | 114 | 2 | 84 | 2 |

[1] mean value of all measured samples per treatment group
RSD = relative standard deviation
n.a = not applicable
LOD = limit of detection (0.003 mg a.s./L)
LOQ = limit of quantification (0.026 mg a.s./L)
* values below the LOQ, shown for information only

**Table 18: The measured concentration of test material and the corresponding geometric mean measured concentrations**

| Nominal concentration (mg test item/L ) | Measured concentration mg a.s./L | | | | Geometric mean measured[1] | |
|---|---|---|---|---|---|---|
| | Day 0, Replicate | | Day 7, Replicate | | | |
| | 1 | 2 | 1 | 2 | mg a.s./L | mg test item/L |
| 1 | 0.06 | 0.059 | 0.013 | 0.013 | 0.028 | 0.54 |
| 3.2 | 0.182 | 0.191 | 0.014 | 0.012 | 0.049 | 0.95 |
| 10 | 0.591 | 0.587 | 0.061 | 0.055 | 0.185 | 3.58 |
| 32 | 1.857 | 1.845 | 0.600 | 0.591 | 1.050 | 20.35 |
| 100 | 5.434 | 5.394 | 3.452 | 3.404 | 4.308 | 83.49 |
| 316 | 17.975 | 17.855 | 13.552 | 12.357 | 15.226 | 295.08 |
| 1000 | 57.815 | 59.09 | 43.789 | 42.514 | 50.218 | 973.22 |

[1] Geometric mean values were not provided in the original report and have been provided during Peer Review process of mepiquat chloride. Geometric mean measured values have been calculated, where test material was detected but was below the limit of quantification, a value of half the limit of quantification was used for the calculation, following methodology defined in OECD Series on testing assessment No. 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures (ENV/JM/MONO(2000)6). It was noted that concentration 10 mg test item/L and below were all <LOQ but the half of the LOQ was used only for the concentrations of 1.0 mg test item/L. The others were within the calibration range and were provided for information purposes only. The geometric mean should be calculated using half of the LOQ, however, this would not change significantly the outcome in this case.

Endpoints have been calculated with the geometric mean measured concentrations using ToxRat Professional Version 3.2.1 (released 2.11.2015).

Frond production and appearance were recorded on days 2, 5, and 7. Dry weight was measured on day 0 (from a sample of similar fronds) and at day 7 in all samples after drying at 60 °C to a constant weight.

Growth in controls met the validity criteria of doubling time < 2.5 days with 1.6 days. Inhibition of yield and growth rate based on frond number was statistically significant from controls at nominal concentration of 3.2 mg test item/L and above. With dry weight as the endpoint, statistically significant inhibition in yield and growth rate was seen at concentrations from 32 mg test item/L (nominal) and above, with significant dip in yield at 3.2 mg test tiem/L (see Table 19 below).

**Table 19: Yield, growth rate and % inhibition of *Lemna gibba* after 7 days exposure to BAS 083 34 W**

| Nominal concentration mg test item/L | Frond number (0 - 7 days) | | | | Dry weight (after 7 days) | | | |
|---|---|---|---|---|---|---|---|---|
| | Yield | % inhibition | Growth rate | % inhibition | Yield | % inhibition | Growth rate | % inhibition |
| Control | 238.0 | - | 0.434 | - | 32.2 | - | 0.475 | - |
| 1.0 | 230.7 | 3.1 | 0.430 | 1.0 | 32.8 | -1.7 | 0.478 | -0.5 |
| 3.2 | 205.8 | 13.9* | 0.414 | 4.7* | 29.8 | 7.5* | 0.464 | 2.3 |
| 10 | 206.7 | 13.0* | 0.415 | 4.4* | 30.4 | 5.7 | 0.467 | 1.7 |
| 32 | 146.3 | 38.5* | 0.368 | 15.1* | 24.4 | 24.4* | 0.437 | 8.1* |
| 100 | 107.0 | 55.0* | 0.328 | 24.5* | 20.6 | 36.2* | 0.414 | 12.9* |
| 316 | 77.0 | 67.6* | 0.286 | 34.0* | 15.8 | 51.1* | 0.378 | 20.4* |
| 1000 | 27.7 | 88.4* | 0.171 | 60.7* | 10.9 | 66.2* | 0.330 | 30.6* |

| Endpoints (mg a.s item/L) based on geometric mean concentration[1] | | | | |
|---|---|---|---|---|
| | Frond number | | Dry weight | |
| | Yield | Growth rate | Yield | Growth rate |
| $EC_{50}$ (7-day) | 2.88 | 31.77 | 13.18 | 50.11 |
| $EC_{20}$ (7-day) | 0.26 | 2.65 | 0.85 | 13.39 |
| $EC_{10}$ (7-day) | 0.07 | 0.73 | 0.21 | 2.26 |
| NOEC | 0.03 | 0.03 | 0.03 | 0.03 |
| LOEC | < 0.03 | 0.05 | n.d. | n.d |

***** Statistically significant ($\alpha = 0.05$)

[1] Data not provided in the report, calculated and provided during Peer Review Process.

95% confidence interval in brackets

n.d. = not determined

Both frond number and dry weight were assessed in this test and frond number was the most sensitive endpoint. Test concentrations of the test item dropped during the test, but the results are based on geometric mean measured concentrations. The $EC_{50}$ (7-d, frond number) based on growth rate and calculated as active substance mepiquat chloride was 31.77 mg a.s./L. The study is considered valid for the classification purpose.

## 11.6 Long-term aquatic hazard

**All available data on chronic aquatic toxicity are summarized in the following table. The new studies submitted to support PPP renewal process of mepiquat chloride are studies on duckweed (*Lemna gibba*) and green algae (*Pseudokirchneriella subcapitata*).**

**Table 720: Summary of relevant information on chronic aquatic toxicity**

| Method | Species | Test material | Results[1] | Remarks | Reference |
|---|---|---|---|---|---|
| **Fish** | | | | | |
| 28 day flow-through OECD 204 GLP | Rainbow trout *(Oncorhynchus mykiss)* | Mepiquat chloride (powder)<br><br>purity 99.9 % | NOEC = 100 mg a.s./L (nom)<br><br>No mortality or sub-lethal effects were seen at any of the tested concentrations and therefore the NOEC was determinded to be 100 mg a.s./L based on nominal concentrations. | Four test concentrations plus control, one replicate with 20 fish per group. Homogeneity of the test compound was verified by analysis. Test concentrations ranged between 95.8 and 112.8 % of nominal throughout the trial. No significant deviations from the guideline. Validity criteria fulfilled. Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014.<br>Supportive study | dRAR B.9.2.1.2. CA 8.2.2 (1993b) |
| 95 day flow-through EPA 72-4(a), OECD 210 GLP | Rainbow trout *(Oncorhynchus mykiss)* | Mepiquat chloride (tech., liquid) purity 660 g/L (nominal), 598.1 g/L (measured) | ELS - NOEC = 100 mg a.s./L (nom) | Six test concentrations plus control. Test concentrations ranged 101.0 – 114.7 % throughout the test. Homogeneity of the test compound was verified by analysis. No significant deviations from the guideline. Validity criteria fulfilled. | dRAR B.9.2.1.3. CA 8.2.2.1 (1995) |
| **Aquatic invertebrates** | | | | | |
| 21 day static-renewal EEC XI/681/86 GLP (equivalent to the OECD 211 test guideline) | *Daphnia magna* | Mepiquat chloride<br><br>purity 99% | NOEC = 12.5 mg a.s. /L (nom)<br>LOEC = 25 mg a.s./L<br>(adult survival) | Eight test concentrations plus control. Ten animals per treatment. Test concentrations ranged between 92.7 and 102.6 % of nominal. Validity criteria fulfilled. Homogeneity of the test compound was verified by analysis. No significant deviations from the OECD 211 guideline. | dRAR B.9.2.2.43 CA 8.2.5.1 (1993) |
| **Algae** | | | | | |
| 72 hrs static OECD 201 GLP | *Pseudokirchneriella subcapitata* | Mepiquat chloride<br><br>purity 99 % | NOEC = 1000 mg a.s./L (nom) | Six test concentrations. 5 replicates per treatment group and 10 in the control. Measured concentrations were between 103.3 and 106.9 % of nominal (tested concentrations were the lowest, mid and | dRAR B.9.2.3.1. CA 8.2.6.1 (1993a) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | highest concentration). Validity criteria of OECD 201 were partially fulfilled: Growth rate in controls fulfilled the criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate control during the whole test fulfilled the criterion < 7 %. The mean coefficient of variation for section-by section specific growth rates exceeded the criterion (41.5% > 35%). | |
| 96 h<br>static<br>ASTM E 1218-90<br>OECD 201<br>EPA 850.1000<br>GLP | *A. flos-aquae* | BAS 083 52 W (617.6 g/L Mepiquat chloride, water based liquid) (nominal 600 g mepiquat chloride/L) | 72 h<br>$E_rC_{10}$ = 4.588 mg a.s./L (nom.) | Five test concentrations plus control. Five replicates per treatment group and 10 replicates for the control. Measured concentrations were 90-109.0 % of nominals (all the concentrations were tested). Validity criteria of OECD 201 were partially fulfilled: Growth rate in controls fulfilled the criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate control during the whole test fulfilled the criterion < 7 % (2.8%). The mean coefficient of variation for section-by section specific growth rates (days 0-2, 2-3, 3-4) exceeded the criterion (64.3% > 35%). However over days 0-2 and 2-3 it was 32.9% meeting requirement of ≤ 35 %. Test is considered valid up to 72 h. | dRAR B.9.2.3.2. CA 8.2.6.2 (2002a) |
| 72 hrs<br>static<br>OECD 201<br>GLP | *Pseudokirchneriella subcapitata* | Mepiquat chloride<br><br>purity 98.1 % | NOEC = 316 mg a.s./L (nom)<br><br>LOEC = 1000 mg a.s./L (nom)<br><br>$E_bC_{10}$ = >429 mg a.s./L (nom)<br><br>$E_rC_{10}$ = >1000 mg a.s./L (nom) | Five test concentrations plus control. Three replicates per treatment group and six in the control. Measured concentrations were between 93.5 and 104.0 % of nominal (all the concentrations were tested).<br><br>The validity criteria of OECD 201 are fulfilled: Increase in cell density was 195.4-fold within 72 hours. Growth rate in controls was 1.763 ± 0.019 1/d fulfilling the the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the | dRAR B.9.2.3.1 CA 8.2.6.1 (2019) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | whole test was 1.3 % fulfilling the validity criterion < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) was 7.2 % fulfilling the validity criterion < 35 %). | |
| **Aquatic plants** | | | | | |
| 7 day static OECD draft guideline (Oct.2000) Lemna sp. growth inhibition test EPA 850.4400 ASTM E 1415-91 GLP | *Lemna gibba* | BAS 083 52 W (617.6 g/L Mepiquat chloride, water based liquid formulation) (nominal 600 g/L) | NOEC = 0.01 mg a.s./L (nom) $E_rC_{10}$ = 0.15 (nom)<br><br>$E_rC_{10}$ = 0.819 mg a.s./L (based on geometric mean measured concentrations which were measeured only from three highest nominal test concentrations 1.0, 10 and 100 mg a.s./L) Endpoint: Frond number | Six test concentrations plus control. 3 replicates per treatment group and 6 for the control. Concentrations were measured from three highest concentration and were 86.4-105.4 % of nominals at the start of the test and 31.6-117.7 % at the end. Test concentrations were unstable at the lowest measured concentrations (1.0 mg a.s./L) were measured concentrations at the end of the study were only 31.6 – 35.6 % of nominal. Statistically significant inhibition was already observed from nominal 0.10 mg a.s./L.. Doubling time of frond number in the control was 2.1 fulfilling the OECD 221 criteria (< 2.5 d). Only frond number was measured.<br><br>**Supportive study for chronic classification** | dRAR B.9.2.4. CA 8.2.7/1 (2003) |
| 7 day static OECD 221 (2006) EC No 761/2009, C.26 (2009) GLP | *Lemna gibba* | BAS 083 34 W (51.6 g/L mepiquat chloride, pinkish liquid) (50.0 g/l mepiquat chloride, nominal) | **$E_rC_{10}$ = 0.73 mg a.s./L**<br>**NOEC 0.03 mg a.s./L (mm)**<br>Endpoint: Frond number growth rate | Doubling time in controls 1.6 days, validity criterion of OECD 221 was met (<2.5 d). Test medium was clear throughout the test. Both frond number and dry weight were assessed, frond number being most sensitive endpoint. The results are based on geometric mean measured concentrations.<br><br>**Key study** | dRAR B.9.2.4. CA 8.2.7/2 (2017) |

mm = mean measured; nom = nominal
Test concentrations expressed as mg a.s./L refer to the concentrations of mepiquat chloride and results are based on the active substance content

### 11.6.1 Chronic toxicity to fish

Two long-term studies with fish were available; one 28-days sublethal test and the one fish early life stage test, both conducted with rainbow trout (see Table 720 above).

The sublethal effects of mepiquat chloride were studied to rainbow trout in 28-days test which was performed according to OECD 204 test guideline and following GLP. It is noted that following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014. No significant deviations from the OECD 204 test guideline were identified. The validity criteria of the guideline were fulfilled (mortality in the control < 10 %, DO > 60 %, test concentrations >80% of the nominal throughout the study). Test concentrations were sufficiently maintained in the test solution (95.8 and 112.8 %). The resulting endpoint of 28 d-NOEC was 100 mg/ a.s./L based on nominal concentrations. However, test is not considered adequate for the chronic classification, therefore, it is used only as a supportive information.

In the **early life stage test** rainbow trout embryos were exposed to six mepiquat chloride (purity 598.1 g/L [measured]) concentrations plus control for 95 days under flow through conditions. The endpoints evaluated were embryo hatching, the percentage of embryo producing live, normal larvae at hatching, larval survival and larval growth. The study was conducted according to OECD 210 guideline and in compliance with GLP. No significant deviations from the test guideline were identified. The validity criteria were fulfilled; the test concentrations were sufficiently maintained throughout the study (mean concentrations were 1.01, 6.87, 13.73, 28.42, 57.36 and 110.73 mg/L corresponding to 101-114.7 % of nominal), dissolved oxygen was > 60 % of saturations, and test temperature maintained generally at 10 C°. Hatchability in controls was > 75 %. No adverse effects were reported throughout the trial, and the NOEC was therefore 100 mg a.s./L, based on nominal concentrations.

Based on the available information mepiquat chloride is not chronicly toxic to aquatic fish.

### 11.6.2 Chronic toxicity to aquatic invertebrates

Only one chronic toxicity test with *Daphnia magna* was available (see Table 720 above).

The chronic toxicity of mepiquat chloride (purity 99 %) to water flea *Daphnia magna* was determined in a 21-day static-renewal test. Mepiquat chloride was tested at nominal concentrations of 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L. The study was conducted according to EEC guideline XI/681/86 (equivalent to OECD 211 test guideline) and following GLP. No significant deviations from the OECD 211 test guideline was apparent. The validity criteria of the test guideline were fulfilled (at the end of the study in controls the mortality of parents did not exceed 20 %, mean number of living offspring 7 parent > 60). The test concentrations were sufficiently maintained during the test (92.7 and 102.6% of nominal). Temperature during the study was 19.6 – 21.0 °C, pH 7.6-8.4 and dissolved oxygen 7.3 – 9.5 mg/L.

Observation on the survival and reproduction were made daily. Adult survival was affected from 25 mg a.s./L with none surviving to reproduce at concentrations above this. There were no adverse effects reported at concentrations below 25 mg a.s./L.

The NOEC for Daphnia magna was 12.5 mg a.s./L, based on nominal concentrations and the LOEC was determined to be 25 mg a.s./L.

### 11.6.3 Chronic toxicity to algae or other aquatic plants

Three algae studies and two studies with aquatic plant *Lemna gibba* were available (see Table 720 above).

*Studies with algae*

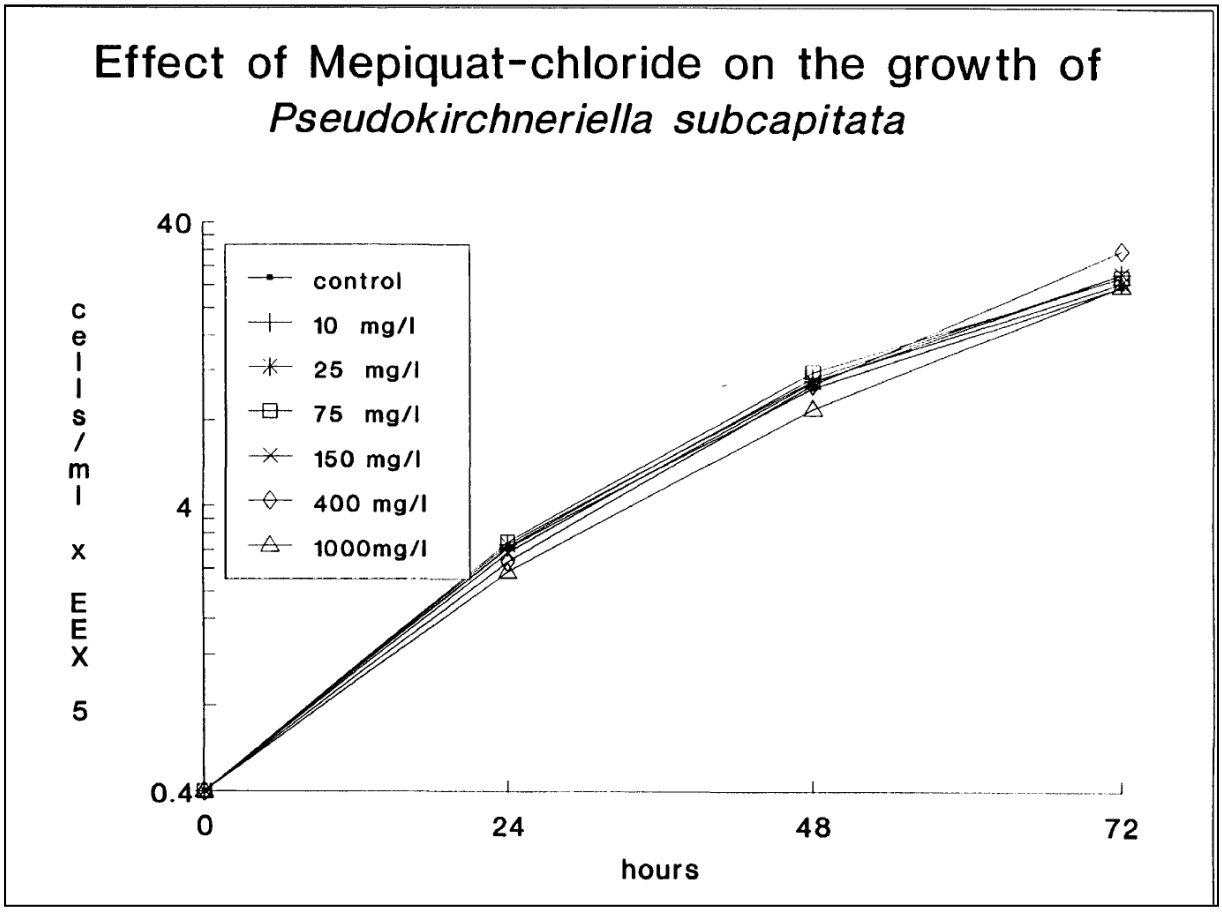
*Study 1 - Pseudokirchneriella subcapitata*

The effect of mepiquat chloride (purity 99%) on the growth of green alga ***Pseudokirchneriella subcapitata*** was determined over a 72-hour exposure period. This study is already summarised in chapter 11.5.3. Concentrations of the test item were confirmed by analysis of treated growth medium at the start and after 72-hours (concentrations tested were 10, 150 and 1000 mg a.s./L) and found to be between 103.3 and 106.0% of nominal. Therefore, the results were based on nominal concentrations. **The NOEC for biomass and growth rate was 1000 mg a.s./L.**

The study was conducted according to OECD 201 guideline following GLP. The validity criteria of OECD 201 were only partially fulfilled: Growth rate in controls was 1.360 ± 0.015 1/d fulfilling the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 1.1 % fulfilling the validity criterion < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) was, however, 41.6 % exceeding the criterion (< 35 %). The average growth rate during 0 – 1, 1- 2 and 2 – 3 days was 2.0, 1.3 and 0.8, respectively. A similar reduction in growth rates is observed for the treatments.

During the Peer Review Process of mepiquat chloride it was noted that "During the 48–72 hour period, growth in all concentrations dropped below the overall mean growth rate and growth rates exceeded that in the controls at the two highest concentrations tested, suggesting that there were very limited effects. The section-by-section growth rate criteria were introduced to ensure that variation within the controls does not undermine the ability to detect toxic effects. It would also identify whether there was a defined lag in growth that could indicate a recovery from a toxic effect with time; neither of the above were evident in this study. Considering that there were very clearly no toxic effects on this organism, the minor exceedance in the section-by-section growth rate of the controls does not impact on the validity of the results." This argument was considered relevant although the validity criteria of OECD 201 were strictly speaking not met. Considering the growth curves (see Figure 5 below) and that no significant inhibition was observed, the minor deviation from the validity criterion can be accepted in this case. Dossier submitter is also in favour of accepting this study and obtained NOEC value of 1000 mg a.s./L for classification purpose.

**Figure 5. The effect of mepiquat chloride on the growth of *Pseudokirchneriella subcapitata***

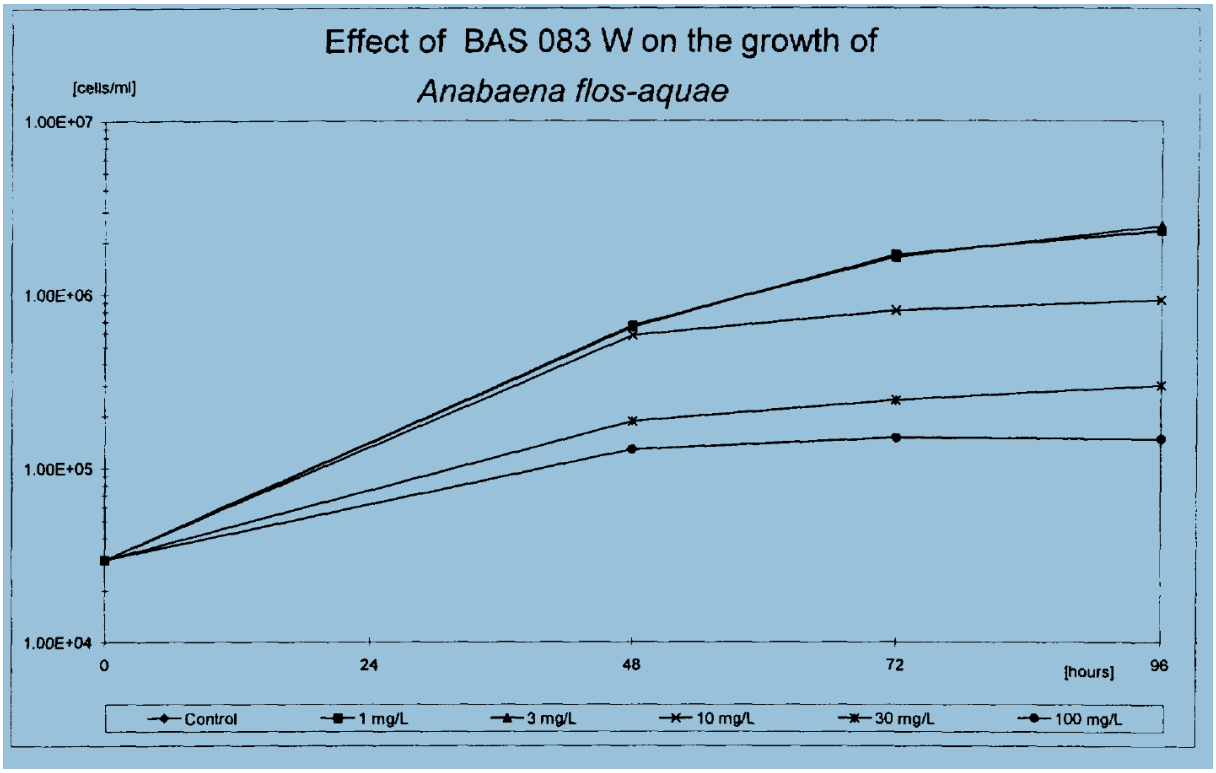



Study 2 - *Anabaena flos-aquae*

The effect of mepiquat chloride (purity 617.6 g mepiquat chloride/L, water based liquid) on the growth of ***Anabaena flos-aquae*** was determined over a 96-hour exposure period. The study is summarised in detail in section 11.5.3. Test concentrations were confirmed by analysis from all concentrations and found to be between 90.0 and 109.0% of nominal at the start of the test and 93.7-107.8 % at the end. The results were therefore based on the nominal concentrations.

The study was conducted according to OECD 201 guideline following GLP. The validity criteria of OECD 201 were partially fulfilled: Growth rate in controls was 1.089 ± 0.016 1/d fulfilling the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 2.8 % fulfilling the validity criterion of < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0 - 2, 2 - 3 and 3 - 4) was, however, 64.3 % exceeding the criterion (< 35 %). The average growth rate during 0 – 2, 2 - 3 and 3 - 4 days was 1.5, 1.0 and 0.3, respectively. A similar reduction in growth rates is observed for the treatments (see Figure 6 below).

**Figure 6. The effect of mepiquat chloride on the growth of *Anabaena flos-aquae***




During the Peer Review Process it was argued that the mean coefficient of variation for the section-by-section growth rate in the controls over days 0-2 and 2-3 was 32.9 % which meets the requirement of ≤ 35%. Considering that a algae study is normally performed up to 72 hours, the study was considered valid up to 3 days (72 hours). The endpoints calculated for 72 hours were provided and are presented in the Table 22. Dossier submitter is in favour of considering this study acceptable up to 72 h and to use obtained $E_rC_{10}$ value of 4.588 mg a.s./L also for classification purpose.

**Table 21: The effect of BAS 083 W on the growth of *Anabaena flos-aquae* - the 72-hours endpoints**

| Test species | Test material | Test System | Endpoint (95% confidence limits) |
|---|---|---|---|
| *Anabaena flos-aquae* | BAS 083 W | 72 - hour | $E_rC_{50}$ = 48.241 (45.574 – 51.176)<br>$E_rC_{20}$ = 10.289 (9.441 – 11.139)<br>**$E_rC_{10}$ = 4.588 (4.052 – 5.137)**<br>$E_bC_{50}$ = 10.048 (9.773 – 10.328) |

| | | | |
|---|---|---|---|
| | | | $E_bC_{20}$ = 4.928 (4.699 – 5.150)<br>$E_bC_{10}$ = 3.396 (3.186 – 3.601) |

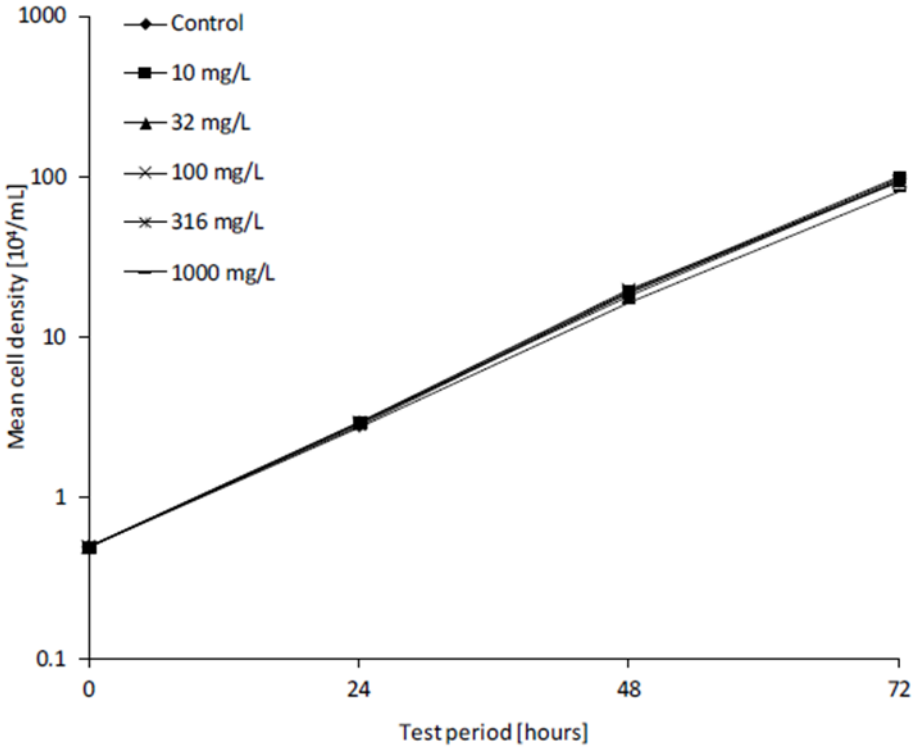
*Study 3 - Pseudokirchneriella subcapitata*

The effect of mepiquat-chloride (purity 98.1%) on the growth of the freshwater green algae, *Pseudokirchneriella subcapitata* was determined over a 72-hour exposure period. This study is already summarised in chapter 11.5.3. Concentrations of the test item were confirmed by analysis of test item at the start and after 72-hours (all the concentrations were tested) and found to be between 93.5 and 104.0% of nominal. Therefore, the results were based on nominal concentrations.

Growth inhibition in the form of biomass and growth rate was assessed after 72 hours. The **$E_bC_{10}$** for **biomass** and the **$E_rC_{10}$** for **growth rate** were **429 mg a.s./L** and >**1000 mg a.s./L**, respectively. Based on the **growth rate** and biomass, the **NOEC** and **LOEC** were **316** and **1000 mg a.s./L**, respectively. Morphological effects on algae were assessed but not observed.

The validity criteria of OECD 201 are fulfilled: Iincrease in cell density was 195.4-fold within 72 hours. Growth rate in controls was 1.763 ± 0.019 1/d fulfilling the the validity criterion of > 0.92 1/day. The coefficient of variation of average specific growth rates in replicate controls during the whole test was 1.3 % fulfilling the validity criterion < 7 %. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) was 7.2 % fulfilling the validity criterion < 35 %). A similar trend was observed in the test concentrations as well (see Figure 7 below).

**Figure 7. The effect of mepiquat chloride on the growth of *Pseudokirchneriella subcapitata***




*Studies with aquatic plant – Lemna gibba*

*Study 1*

The effect of BAS 083 52 W (mepiquat chloride 617.6 g/L, water based liquid formulation) on the growth of the duckweed ***Lemna gibba*** was determined over a 7 day exposure period (nominal concentrations were 0.001, 0.01, 0.1, 1.0, 10.0 and 100 mg a.s./L). The study is summarised in detail in the chapter 11.5.3.

The study was performed according to the draft OECD 221 test guideline following GLP. The concentration analyses were taken from the three highest test concentrations 1, 10.0 and 100 mg a.s./L (nominal). Measured were in a range of 86.4 – 105.4% of nominal at the start of the test, and between 31.6 – 117.7% of nominal at the end (at 1.0 mg a.s./L only 31.6 – 35.6 % of nominal at the end) (see Table 22).

**Table 22: Measured concentrations of mepiquat chloride in the exposure solutions**

| Nominal concentration (mg a.s./L) | Mean measured concentration (mg a.s./L) | | | | Geometric mean measured (mg a.s/L) |
|---|---|---|---|---|---|
| | 0-Day | % Nominal | 7-Day | % Nominal | |
| 1 | 1.0 | 99.8 | 0.32 | 31.6 | 0.57 |
| 1 | 0.97 | 97.4 | 0.36 | 35.7 | |
| 10 | 10.24 | 102.4 | 10.24 | 102.4 | 10.25 |
| 10 | 10.32 | 103.2 | 10.15 | 101.5 | |
| 100 | 86.36 | 86.4 | 97.02 | 97.0 | 100.94 |
| 100 | 105.39 | 105.4 | 117.67 | 117.7 | |

In the test report the results were based on nominal concentrations, the NOEC for growth rate was determined to be 0.01 mg a.s./L and $E_rC_{10}$ was 0.15 mg a.s./L (95% confidence interval 0.12 – 0.2) (see Table 23). Because unstable exposure conditions were observed at low mepiquat concentration (1.0 mg a.s./L), the results were also calculated based on geometric means of the concentrations in the three highest exposure concentrations (nominal 1, 10 and 100 mg/l; measured geom. mean: 0.57; 10.25 and 100.94 mg/l) resulting; $EC_{20}$ of 2.341 and $EC_{10}$ of 0.819 mg a.s./L. It is noted that statistically significant effects were observed already at the nominal concentration of 0.10 mg/L where no measurements were available. The NOEC of 0.01 mg a.s./L and $E_rC_{10}$ of 0.15 mg a.s./L (based on nominal concentrations) are then related with some uncertainty, as actual effect concentrations might be even lower.

**Table 23: Percentage growth inhibition of *Lemna gibba* after 7 days exposure to mepiquat chloride**

| Nominal concentration (mg a.s./L) | % Inhibition in 7 days | |
|---|---|---|
| | Growth rate | Frond number |
| 0.001 | 0.76 | 0.30 |
| 0.01 | 5.66 | 10.98 |
| 0.10 | 12.75* | 28.35* |
| 1.0 | 18.50* | 38.72* |
| 10.0 | 32.11* | 58.23* |
| 100 | 83.36* | 94.82* |
| **Endpoints (mg a.s./L)** | | |
| $E_rC_{50}$ (7-day) | 15.41 (13.53 – 17.56[1]) | |
| $E_rC_{10}$ (7-day) | 0.15 (0.12 – 0.2[1]) | |
| $E_bC_{50}$ (7-day) | 2.6 (2.28 – 2.96[1]) | |
| $E_bC_{10}$ (7-day) | 0.01 (0.01 – 0.02[1]) | |
| NOEC | 0.01 | |
| LOEC | 0.01 | |

* Statistically significant ($\alpha = 0.05$)
[1] 95% confidence interval

Another deviation (in addition to that the results were initially based on nominal concentrations although at 1 mg/l the measured concentrations were unstable) from the OECD 221 test guideline was that only frond number was measured. As according to the test guideline at least one other measurement variable (total frond area, dry weight or fresh weight) should also be measured since some substances may affect other measurement variables much more than frond numbers. There is another Lemna study (Study 2) available where both frond number and dry weight were measured and the frond number was the most sensitive endpoint. Therefore, it might be considered that the lack of another variable is not devalidating this study, keeping in mind that the test substances are not completely the same in these studies.

It might be argued that as the measured concentrations did not stay ±20 % of nominals results should be based on the geometric mean measured concentrations. However, they were measured only from the three highest test concentrations and statistically significant effects were already seen at lower dose (0.1 mg a.s./L) where no analytics were available.

Both NOEC and $E_rC_{10}$ values are available, and in that case $E_rC_{10}$ value is usually preferred over NOEC. $E_rC_{10}$ value of 0.819 mg a.s./L based on geometric mean measured concentrations is in same order of magnitude as $E_rC_{10}$ value of 0.15 mg a.s./L based on nominal concentrations. However, there remains some uncertainty that the effect concentration might be even lower as analyses from three concentrations revealed that the mepiquat chloride dissipates at low concentrations to a large extent (1.0 mg a.s./L), but at higher concentrations it seems to be more stable.

As a conclusion dossier submitter is proposing to use this study as a supportive study for chronic classification of mepiquat chloride as no definitive conclusion of reliable effect concentrations for chronic hazard can be drawn.

*Study 2*

The other Lemna study confirms the high chronic toxicity of mepiquat chloride to aquatic plant *Lemna gibba*. The effect of BAS 083 34 W (mepiquat chloride 51.6 g/L, pinkish liquid) on the growth of the duckweed *Lemna gibba* was determined in a static test over a 7-day exposure period. Test was conducted

according to OECD 221 and EC No 761/2009, C26 (2009) guidelines and in compliance with GLP. Test item BAS 083 34 W is noted to contain only one co-formulant in very low concentration which do not have harmonised or self-classification as hazardous to the aquatic environment. The rest of the test item is water. The detailed content of the test item is provided in the confidential annex. The results are based on the active substance mepiquat chloride content. Summary of the test method is provided in the chapter 11.5.3.

Growth in controls met the validity criteria of doubling time < 2.5 days with 1.6 days. Both frond number and dry weight were assessed, frond number being the most sensitive endpoint. Inhibition of yield and growth rate based on frond number was statistically significant from controls at nominal concentration of 3.2 mg test item/L and above. With dry weight as the endpoint, statistically significant inhibition in yield and growth rate was seen at concentrations from 32 mg test item/L (nominal) and above, with significant dip in yield at 3.2 mg test tiem/L (see Table 24).

**Table 24: Yield, growth rate and % inhibition of *Lemna gibba* after 7 days exposure to BAS 083 34 W**

| Nominal concentration mg test item/L | Frond number (0 - 7 days) | | | | Dry weight (after 7 days) | | | |
|---|---|---|---|---|---|---|---|---|
| | Yield | % inhibition | Growth rate | % inhibition | Yield | % inhibition | Growth rate | % inhibition |
| Control | 238.0 | - | 0.434 | - | 32.2 | - | 0.475 | - |
| 1.0 | 230.7 | 3.1 | 0.430 | 1.0 | 32.8 | -1.7 | 0.478 | -0.5 |
| 3.2 | 205.8 | 13.9* | 0.414 | 4.7* | 29.8 | 7.5* | 0.464 | 2.3 |
| 10 | 206.7 | 13.0* | 0.415 | 4.4* | 30.4 | 5.7 | 0.467 | 1.7 |
| 32 | 146.3 | 38.5* | 0.368 | 15.1* | 24.4 | 24.4* | 0.437 | 8.1* |
| 100 | 107.0 | 55.0* | 0.328 | 24.5* | 20.6 | 36.2* | 0.414 | 12.9* |
| 316 | 77.0 | 67.6* | 0.286 | 34.0* | 15.8 | 51.1* | 0.378 | 20.4* |
| 1000 | 27.7 | 88.4* | 0.171 | 60.7* | 10.9 | 66.2* | 0.330 | 30.6* |

| Endpoints (mg a.s item/L) based on geometric mean concentration[1] | | | | |
|---|---|---|---|---|
| | Frond number | | Dry weight | |
| | Yield | Growth rate | Yield | Growth rate |
| $EC_{50}$ (7-day) | 2.88 | 31.77 | 13.18 | 50.11 |
| $EC_{20}$ (7-day) | 0.26 | 2.65 | 0.85 | 13.39 |
| $EC_{10}$ (7-day) | 0.07 | 0.73 | 0.21 | 2.26 |
| NOEC | 0.03 | 0.03 | 0.03 | 0.03 |
| LOEC | < 0.03 | 0.05 | n.d. | n.d |

***** Statistically significant ($\alpha = 0.05$)

[1] Data not provided in the study report, calculated and provided during Peer Review Process.

95% confidence interval in brackets

n.d. = not determined

Test concentrations of the test item dropped during the test, but the results are based on geometric mean measured concentrations (see Table 251) and calculated as active substance mepiquat chloride. The NOEC was determined to be 0.03 mg a.s. /L and $E_rC_{10}$ 0.73 mg a.s./L (7-d, frond number, growth rate). This study is considered valid for classification purpose. When both values NOEC and $EC_{10}$ are available, $EC_{10}$ is usually preferred over NOEC. **The obtained $E_rC_{10}$ value of 0.73 mg a.s./L is used to derive chronic classification of mepiquat chloride.**

**Table 251: The measured concentration of test material and the corresponding geometric mean measured concentrations**

| Nominal concentration (mg test item/L ) | Measured concentration mg a.s./L | | | | Geometric mean measured[1] | |
|---|---|---|---|---|---|---|
| | Day 0, Replicate | | Day 7, Replicate | | | |
| | 1 | 2 | 1 | 2 | mg a.s./L | mg test item/L |
| 1 | 0.06 | 0.059 | 0.013 | 0.013 | 0.028 | 0.54 |
| 3.2 | 0.182 | 0.191 | 0.014 | 0.012 | 0.049 | 0.95 |
| 10 | 0.591 | 0.587 | 0.061 | 0.055 | 0.185 | 3.58 |
| 32 | 1.857 | 1.845 | 0.600 | 0.591 | 1.050 | 20.35 |
| 100 | 5.434 | 5.394 | 3.452 | 3.404 | 4.308 | 83.49 |
| 316 | 17.975 | 17.855 | 13.552 | 12.357 | 15.226 | 295.08 |
| 1000 | 57.815 | 59.09 | 43.789 | 42.514 | 50.218 | 973.22 |

[1] Geometric mean values were not provided in the original report and have been provided during Peer Review process of mepiquat chloride. Geometric mean measured values have been calculated, where test material was detected but was below the limit of quantification, a value of half the limit of quantification was used for the calculation, following methodology defined in OECD Series on testing assessment No. 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures (ENV/JM/MONO(2000)6). It is noted that concentration 10 mg test item/L and below were all <LOQ but the half of the LOQ was used only for the concentrations of 1.0 mg test item/L. The others were within the calibration range and were provided for information purposes only. The geometric mean should be calculated using half of the LOQ, however, this would not change significantly the outcome in this case.

Endpoints have been calculated with the geometric mean measured concentrations using ToxRat Professional Version 3.2.1 (released 2.11.2015).

### 11.6.4 Chronic toxicity to other aquatic organisms

## 11.7 Comparison with the CLP criteria

### 11.7.1 Acute aquatic hazard

Full acute data set was available for mepiquat chloride as there were acute toxicity studies on fish, aquatic invertebrates, algae and aquatic macrophytes. All acute effect values were above 1 mg/L. The most sensitive species were eastern oyster *Crassostrea virginica* with an $EC_{50}$ value of 15 mg a.s./L (shell growth) and *Lemna gibba* with an $EC_{50}$ value of 17.45 mg a.s./L (growth rate, frond number). Based on the available data it is concluded that **mepiquat chloride does not fulfil the criteria for classification as Aquatic Acute Category 1** (≤ 1 mg/l) according to the CLP.

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

*Bioaccumulation*

No experimental BCF studies on mepiquat chloride are available. The study on partition coefficient n-octanol/water (OECD test guideline 107 "Shake Flask Method") resulted in log $P_{OW}$ values from -3.14 to -3.55. This is less than the trigger value of 4 given in the CLP Regulation. Although octanol cannot be used as a surrogate of lipid sorption for a surface-active substance, there is no indication of the substance being bioaccumulative. Therefore, **the substance is considered to have low potential to bioaccumulate for the classification purposes**.

*Rapid degradation*

According to a **ready biodegradability test** (OECD test guideline 301A "DOC Die-Away"), 90-100% of mepiquat chloride degradated after 28 days. As the pass level criteria of ready biodegradation test (70% of DOC removal) was reached in a 10-day window within 28 days period, the test suggests mepiquat chloride being rapidly degradable for purposes of classification.

Based on the **simulation test in surface water** (OECD test guideline 309 "Simulation biodegradation test"), virtually no degradation of mepiquat chloride was observed in natural surface water systems. As the substance is not degraded with a half-life of < 16 days, the CLP criteria for rapid degradation is not fulfilled.

According to a **hydrolysis test** (US EPA guideline: Pesticide Assessment Guidelines: "Hydrolysis studies") mepiquat chloride is hydrolytically stable as no degradation of mepiquat chloride was observed at pH 3, 5, 7 or 9 during the test. According to the criteria in CLP guidance, the substance might be considered as rapidly degradable for classification purposes only when the longest half-life determined within the pH range of 4-9 is shorter than 16 days (and the hydrolysis products formed do not fulfil the classification criteria as hazardous for aquatic environment). Mepiquat chloride does not fulfil this criteria of rapid degradation**.**

The studies on **degradation** in **water/sediment systems** ($DT_{50}$ values of 32.0 and 32.6 days) and in **soil** ($DT_{50}$ values from 3.6 to 35.5 days) support the observations of mepiquat chloride unlikely being rapidly degradable. Furthermore, **photodegradation** of mepiquat chloride was measured being insignificant in **water** and **soil**. However, mepiquat chloride entering the air is subject to rapid indirect photochemical degradation ($DT_{50}$ value of 4.6 hours).

The results from most of the studies and the ready biodegradability study are somewhat contradictory. However, it is possible that e.g. in the simulation test in surface water test, no bacteria that can degrade the test substance are present while in the readily biodegradability test there are sufficient biomass present to degrade the test substance. With regard to the CLP criteria, the degradation information from the ready biodegradability test available provides sufficient data mepiquat chloride being ultimately degraded to > 70% within 28 days (equivalent to a half-life of less than 16 days). Therefore, **mepiquat chloride is considered being rapidly degradable**.

*Toxicity*

Mepiquat chloride is considered being rapidly degradable according to the CLP criteria. The adequate chronic toxicity data for mepiquat chloride was available for three trophic levels fish, aquatic invertebrates, algae and aquatic plants. The lowest endpoint values were for fish NOEC of 100 mg a.s./L (*Oncorhynchus mykiss*), for aquatic invertebrate NOEC of 12.5 mg a.s./L (*Daphnia magna*), for algae 72 h $E_rC_{10}$ of 4.588 mg a.s/L (*Anabaena flos-aquae*), and for aquatic plant *Lemna gibba* $E_rC_{10}$ of 0.73 mg a.s./L (growth rate), which was the most sensitive species.

Mepiquat chloride has already harmonised classification **Aquatic Chronic Category 3**. Based on the available data current classifications based on Table 4.1.0 (b)(ii) of the CLP Regulation remains (rapidly degradable substances for which there are adequate chronic toxicity data available and chronic NOEC or $EC_X \leq 1$ mg/l).

## 11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Based on the CLP Regulation (1278/2008) and available data, classification of mepiquat chloride should remain as:

| | |
|---|---|
| Classification categories | Aquatic Chronic Category 3 |
| Hazard Statement | H412 'Harmful to aquatic life with long lasting effects.' |

## RAC evaluation of aquatic hazards (acute and chronic)

### Summary of the Dossier Submitter's proposal

Mepiquat chloride is the ISO common name for 1,1-dimethylpiperidinium chloride (IUPAC), a quaternary ammonium plant growth regulator. Mepiquat chloride acts as an inhibitor of the biosynthesis of gibberellic acid, absorbed and translocated throughout the plant. This substance is used on cereals to reduce unwanted longitudinal shoot growth without lowering plant productivity. The hazard classification of mepiquat chloride was agreed in the November 1995 meeting of the Commission Working Group on the C&L of Dangerous Substances under Directive 67/548/EEC. The Group agreed to the classification as: Xn; R22-52/53, corresponding to Aquatic Chronic 3 under the CLP regulation.

The conclusion regarding the peer review of the pesticide risk assessment of the active substance Mepiquat was published as an EFSA Scientific Report in 2008. The Renewal Assessment Report (2018) under Regulation (EC) 1107/2009 was used as the main data source for drafting the CLH report of mepiquat chloride.

The Dossier Submitter (DS) presented available relevant data for degradation, bioaccumulation, and all the three trophic levels for acute and chronic aquatic toxicity. Based on this dataset, the DS proposed to keep the existing harmonized classification as **Aquatic Chronic Category 3 H412 Harmful to aquatic life with long lasting effects**.

The water solubility of mepiquat chloride in pure water at 20 °C has been experimentally determined to be 674 g/L at pH 6.1 (OECD TG 105, CIPAC MT181, equivalent to EEC Method A6). Mepiquat chloride completely dissociates in aqueous solutions and therefore has no dissociation constant (dRAR B.2.8/01, 2002). Mepiquat chloride is surface active with a surface tension value of 47.4 mN/m at 20 °C. Based on the laboratory study indicating very low vapour pressure (< 1 × $10^{-8}$ Pa, 20 °C) and theoretical estimation of low Henry´s law constant (3.0 x $10^{-12}$ Pa $m^3$ $mol^{-1}$), mepiquat chloride is considered as non-volatile.

***Degradation***

A brief summary of reliable valid studies considering the aquatic fate of Mepiquat chloride listed in the Draft Renewal Assessment Report (dRAR) is presented by the DS in Table 65, page 88 of the CLH report.

The DS reported a ready biodegradability study (**B.8.3.2.1/01**, **2003**) available in the dRAR, considered as the key study. The test followed OECD TG 301A. Duplicate mixtures of test substance were tested at 62.6 mg/L (lower concentration than the water solubility of mepiquat chloride, > 700 g/L) in a defined inorganic medium and a non-preadapted inoculum. In addition, two blank controls, reference substance, inhibition control (inhibition of the inoculum by test substance), abiotic control and absorption onto the inoculum were tested in parallel. The degree of biodegradation was calculated by expressing the concentration of DOC removed as a percentage of the concentration initially present. The lag-phase for the degradation was 19 days (before the 10% of degradation was reached). After that, the degradation accelerated resulting to 90-100% degradation within 10 days. Degradation of in the inhibitory test vessel was 40-50% DOC after 14 days. As degradation of mepiquat chloride was higher (100%) than the trigger value of 70% within 28 days for

the method, the DS considered that mepiquat chloride fulfilled the criteria to pass the test within the 10 day window as a readily biodegradable substance.

One study on hydrolytic degradation for mepiquat chloride was available in the dRAR and as no degradation of mepiquat chloride was observed over a 30-day period. The DS considered the mepiquat chloride as hydrolytically stable.

The DS presented the aerobic mineralisation of mepiquat chloride in surface water according the OECD TG 309. In conclusion, mepiquat chloride was found to be stable, or degrading only very slowly under the conditions of the test.

One study on the route and rate of degradation of mepiquat chloride in water/sediment systems under aerobic conditions was reported in the dRAR and the CLH report. The study basically followed the OECD TG 308. Mepiquat chloride was found to dissipate relatively rapidly from water, with the major route of dissipation being partitioning to sediment from the water phase. Further kinetic evaluation of the dissipation of mepiquat chloride was reported according to FOCUS Degradation Kinetics Report (2006, 2014). Based on these results, the degradation of mepiquat chloride is considered to be not rapid in natural environments.

Three studies of degradation in soil under aerobic conditions for mepiquat chloride were considered valid in the dRAR and reported by the DS. Two of the studies (B.8.1.1.1/02, 2003 & B.8.1.1.1/03, 1996) were basically performed according the OECD TG 307 and one (B.8.1.1.1/04, 1979) of them didn't follow any guidelines. Based on these results, mepiquat chloride doesn't degrade rapidly in soil under aerobic conditions.

One study (B.8.3.1.2/02, 1990) on photochemical degradation in water for mepiquat chloride conducted generally according to the OECD TG 316 was available in the dRAR and presented by the DS. No photodegradation occurred after several days of continuous exposure indicating aqueous photolysis not being a significant route of degradation of mepiquat chloride in the environment.

Despite the low degradation observed in the environment, as the substance exhibited ready biodegradability under OECD TG 301, **the DS concluded that mepiquat chloride is rapidly degradable for classification and labelling purposes**.

***Bioaccumulation***

The DS reported that an octanol:water partition coefficient was determined following the shake flask method (OECD TG 107) at pH 4, 7 and 10 at 20°C. The quoted log Pow values were – 3.2, -3.55 and -3.14 at pH 4, 7 and 10 respectively. The DS recognised that the shake flask method is not applicable to surface active substances. However, despite the fact that octanol cannot be used as a surrogate for lipid sorption of a surface-active substance, the DS considered that there is no indication of the substance having a high bioaccumulation potential. Based on these log Pow values below the CLP threshold of 4, mepiquat chloride was considered **to have a low bioaccumulation potential**.

***Aquatic toxicity***

The DS noted that some of the ecotoxicological studies were conducted with the technical concentration containing 615-665 g/L mepiquat chloride or with the concentration even

lower (51.6 g/L). However, the test concentrations and results are expressed as mg a.s./L referring to the concentrations of mepiquat chloride.

Acute aquatic hazard

The relevant available data on acute aquatic toxicity are summarized in the table 12 page 98 in the CLH report. Test concentrations expressed as mg a.s./L refer to the concentrations of mepiquat chloride and results are based on the active substance content.

The DS noted that relevant acute toxicity studies are available for the three trophic levels.

Acute fish toxicity studies conducted according to OECD TG 203 following GLP and no significant deviations identified from the test guideline, are available on three fish species (rainbow trout, bluegill sunfish and sheepshead minnow). In the study with rainbow trout, two control fish died at 96 hours, exceeding the OECD TG 203 validity criterion (mortality < 10% in controls). However, as there were no mortalities in any other group this was not considered critical. The DS observed that there was no mepiquat chloride related toxicity in either of these studies and 96 hr-$LC_{50}$ values were > 100 mg a.s./L, > 100 mg a.s./L. and 151 mg a.s./L respectively, and concluded that based on the available studies mepiquat chloride is not acutely toxic to fish up to the maximum concentration tested.

For invertebrates, two acute studies with *Daphnia magna* (according to OECD TG 202, without any significant deviations), one with saltwater mysid *Mysidopsis bahia* (according to US EPA 72-3(b) TG (equivalent US EPA OCSPP 850.1035), with no significant deviation) and one with eastern oyster *Crassotrea virginica* (performed according to US EPA 72-38(c) TG (equivalent to OCSPP 850.1025)) were quoted by the DS. The $EC_{50}$ values of 68.5 mg a.s./L and 106 mg a.s./L were determined for the water fleas, $LC_{50}$ values at 24, 48, 72 and 96 hours were all > 136 mg a.s./L. with saltwater mysid and the estimation of $EC_{50}$ value which was determined to be 15 mg a.s./L, the lowest effect value for acute toxicity, for the oyster.

For primary producers, three algae studies and two studies with aquatic plant *Lemna gibba* were available. The effect of mepiquat chloride (purity 99%) on the growth of green alga *Pseudokirchneriella subcapitata* was determined in two studies over a 72-hour exposure period. In the first study there was no inhibition of algal growth rate as a result of exposure to mepiquat chloride and, based on nominal concentrations, the $E_bC_{50}$ (biomass) and $E_rC_{50}$ (growth rate) both were > 1000 mg a.s./L. The second study fulfilled all validity criteria of OECD TG 201 also confirmed no effects up to 1000 mg/L.

The effect of mepiquat chloride (purity 617.6 g mepiquat chloride/L) was determined over a 96-hour exposure period with the blue-green algae *Anabaena flos-aquae*. Based on nominal concentrations, the $E_bC_{50}$ was 14.4 mg a.s./L (95% confidence interval 13.7 – 15.2) and $E_rC_{50}$ was 44.8 mg a.s./L (95% confidence interval 41.5 – 48.3). The study was conducted according to OECD TG 201 following GLP. The validity criteria of OECD TG 201 were partially fulfilled. The DS reported that during the Peer Review Process it was noted that the mean coefficient of variation for the section-by-section growth rate in the controls over days 0-2 and 2-3 was 32.9% which meets the requirement of ≤ 35%. Considering that algae studies are normally performed up to 72 hours, the study was considered valid up to 3 days (72 hours). The endpoints calculated for 72 hours ($E_rC_{50}$ = 48.241 mg a.s./L) were provided. The Dossier Submitter is in favor of considering this study acceptable up to 72 h also for classification purpose.

The DS reported two toxicity studies conducted with duckweeds. In the first study, an $E_rC_{50}$ value of 17.45 mg/L was derived based on geometric mean measured concentrations and in the second one a measured $E_rC_{50}$ value of 31.77 mg/L. More information on these two studies can be found in the CLH report and Background Document (BD).

The DS concluded that a full acute dataset was available for mepiquat chloride as there were acute toxicity studies on fish, aquatic invertebrates, algae and aquatic macrophytes and all acute effect values were above 1 mg/L. The most sensitive species was eastern oyster *Crassostrea virginica* with an $EC_{50}$ value of 15 mg a.s./L based on shell growth and *Lemna gibba* with an $EC_{50}$ value of 17.45 mg a.s./L (growth rate, frond number). Based on the available data, it is concluded by the DS that **mepiquat chloride does not fulfil the criteria for classification as Aquatic Acute Category 1 (≤ 1 mg/L) according to the CLP**.

Chronic aquatic hazard

The valid available data on chronic aquatic toxicity described by the DS are presented in the table 20 page 111 of the CLH report. Test concentrations expressed as mg a.s./L refer to the concentrations of mepiquat chloride and results are based on the active substance content. More details on the chronic studies can be found in the CLH report and Background Document (BD).

Two long-term studies with fish were presented in the CLH report; one 28-days sublethal test and one fish early life stage test, both conducted with rainbow trout. The first test was only considered by the DS as supportive information as it was performed according to OECD TG 204 that is not considered as equivalent of a chronic test, for classification and labelling purposes. The second study was conducted according to OECD TG 210 and in compliance with GLP reported no adverse effects were reported throughout the test duration and the NOEC was, therefore, 100 mg a.s./L, based on nominal concentrations.

The DS reported only one available chronic toxicity test with *Daphnia magna*. The study was conducted according to EEC guideline XI/681/86 (equivalent to OECD TG 211) and following GLP. No significant deviations from the OECD TG 211 were apparent and the validity criteria of the guideline were fulfilled. The NOEC for *Daphnia magna* was 12.5 mg a.s./L, based on nominal concentrations and the LOEC was determined to be 25 mg a.s./L.

The DS reported that three algae studies and two studies with aquatic plant *Lemna gibba* were available. The most conservative value derived from a study on *Lemna gibba* where the NOEC was determined to be 0.03 mg a.s. /L and the $E_rC_{10}$ was 0.73 mg a.s./L (7-d, frond number, growth rate). When both values NOEC and $EC_{10}$ are available, $EC_{10}$ is usually preferred over NOEC. The obtained **$E_rC_{10}$ value of 0.73 mg a.s./L** was used by the DS to derive chronic classification of mepiquat chloride. The proposal was that the substance is warranted a chronic classification as **Aquatic Chronic 3, H412**.

## Comments received during consultation

Only one comment was received during the consultation: this MS supported the classification proposed by the DS (Aquatic Chronic 3).

## Assessment and comparison with the classification criteria

***Aquatic Acute classification***

Full acute dataset (fish, aquatic invertebrates, algae and aquatic macrophytes) is available for mepiquat chloride. The most sensitive species are eastern oyster *Crassostrea virginica* with an $EC_{50}$ value of 15 mg a.s./L (shell growth) and *Lemna gibba* with an $EC_{50}$ value of 17.45 mg a.s./L (growth rate, frond number). Based on the available data, RAC supports the proposal of the DS and concludes that **mepiquat chloride does not fulfil the criteria for classification as Aquatic Acute Category 1** (≤ 1 mg/L) according to the CLP Regulation.

***Aquatic Chronic classification***

Rapid degradation

In an OECD TG 301A study, mepiquat chloride was considered readily biodegradable, as the pass level criteria of the ready biodegradation test (70% of DOC removal) was reached in a 10-day window within a 28 days period. This GLP-compliant test was performed over 35 days with the end of the lag phase, corresponding to the time when the degradation rate exceeded 10%, being reached in 19 days (see analytical text and associated Figure in the BD section below). RAC considers this study as valid, indicating that according to the CLP criteria mepiquat chloride should be considered readily biodegradable for classification purposes.

Such rapid degradation was not observed in the simulation studies:

- No mepiquat chloride degradation was observed in a surface water simulation study (OECD TG 309). In this case, as the substance is not degraded with a half-life of < 16 days, the CLP criteria for rapid degradation are not fulfilled;
- In the water/sediment simulation (OECD TG 308) and soil studies, the obtained $DT_{50}$ values of 32.0 and 32.6 days, and from 3.6 to 35.5 days respectively support the observations that mepiquat chloride is unlikely to rapidly biodegrade. The removal occurred due to partitioning from the water phase to sediment where mepiquat chloride was absorbed, degraded or bound to this matrix.

Furthermore, according to the hydrolysis test (US EPA guideline: Pesticide Assessment Guidelines: "Hydrolysis studies") mepiquat chloride is hydrolytically stable as no degradation of mepiquat chloride was observed at pH 3, 5, 7 or 9 during the test. This result does not fulfil the criteria of rapid degradation because, according to the criteria in the CLP guidance, the substance might be considered as rapidly degradable for classification purposes only when the longest half-life determined within the pH range of 4-9 is shorter than 16 days (and the hydrolysis products formed do not fulfil the classification criteria as hazardous for aquatic environment).

RAC notes the limited mineralisation observed in the surface water and water/sediment simulation studies, indicating low degradation rates in water. Despite this scientific evidence, RAC has applied the criteria presented in section 4.1.2.9.5. of CLP to conclude that the substance is **rapidly degradable**. This is based on paragraph (a) of that section and the presence of a positive result from a valid and reliable experimental study on ready biodegradability, conducted with an appropriate test protocol.

***Bioaccumulation***

No experimental BCF studies on mepiquat chloride are available. The study on partition coefficient n-octanol/water (OECD TG 107) resulted in log $P_{OW}$ values from -3.14 to -3.55. **RAC noted that the shake flask method is not adequate for surface active**

**substance and in general leads to an underestimation of the log $P_{ow}$ values**. Nevertheless, the obtained value is orders of magnitude below the trigger value of 4 given in the CLP Regulation. Additionally, the molecular structure of this substance indicates that mepiquat chloride is unlikely to be bioaccumulative. Therefore, the substance can be considered to have low potential to bioaccumulate for classification purposes, despite any remaining uncertainties.

***Chronic toxicity***

Chronic toxicity data are available for three trophic levels fish, aquatic invertebrates, algae and aquatic plants. RAC considers the most sensitive species to be aquatic plant *Lemna gibba* $E_rC_{10}$ of 0.73 mg a.s./L (growth rate), compared with the fish NOEC of 100 mg a.s./L (*Oncorhynchus mykiss*), aquatic invertebrate NOEC of 12.5 mg a.s./L (*Daphnia magna*), and algae 72 h $E_rC_{10}$ of 4.588 mg a.s/L (*Anabaena flos-aquae*).

Since adequate chronic toxicity data available, NOEC or $EC_X$ is in the range from 0.1 to 1 mg/L and mepiquat chloride is rapidly degradable, according to table 4.1.0 (b)(ii) of CLP, **RAC agrees with the DS that classification as Aquatic Chronic 3 is warranted.**

## Supplemental information - In depth analyses by RAC

The DS reported two toxicity studies conducted with duckweeds. In the first study, the effect of BAS 083 52 W (mepiquat chloride 617.6 g/L, water based liquid formulation) on the growth of the duckweed Lemna gibba was determined over a 7-day exposure period with the following tested nominal concentration of 0.001, 0.01, 0.10, 1.0 10.0, and 100 mg a.s./L. The study was performed according to the draft OECD TG 221 following GLP with 3 replicates per treatment group and 6 replicates for the control containing 3 fronds from 4 plants. Test vessels were inoculated with less than 10-day old Lemna. Growth inhibition in the form of frond number and growth rate was assessed after 7 days. Concentrations were confirmed by chemical analysis of treated medium at the start of the test and after 7-days. Concentration analyses were taken from three highest test concentrations 1, 10.0 and 100 mg a.s./L as these were above the LOQ. Analyses confirmed test concentrations were in a range of 86.4 – 105.4% of nominal at the start of the test, and between 31.6 – 117.7% of nominal at the end. Temperature during the study was 24-26 °C and pH 8.42-8.51. With fronds increasing from 12 to 121 per vessel corresponding to a doubling time of 2.1 days (10.1 multiplication) the growth requirement in the OECD TG 221 (doubling time of less than 2.5 days) was fulfilled. Statistically significant inhibition was observed at 0.10 mg a.s./L nominal concentration. At a concentrations of 10 mg a.s./L, a reduced ability in separating frond was reported. At 100 mg/L, only single fronds were observed (no colonies). In the test report the results were based on nominal concentrations, the 7-day EbC50 was 2.6 mg a.s./L (95% confidence interval 2.28 – 2.96) and ErC50 was 15.41 mg a.s./L (95% confidence interval 13.53 – 17.56). Because unstable exposure conditions were observed at low mepiquat concentration (1.0 mg a.s./L), the results were also calculated based on geometric means of the concentrations in the three highest exposure concentrations (nominal 1, 10 and 100 mg/L; measured geom. mean: 0.57; 10.25 and 100.94 mg/L) resulting; ErC50 value of 17.45 mg a.s./L.

Another deviation (in addition to that the results were initially based on nominal concentrations although at 1 mg/L the measured concentrations at the end of the study were only 31.6 – 35.6% of nominal) from the OECD TG 221 was that only frond number

was measured. As according to the TG at least one other measurement variable (total frond area, dry weight or fresh weight) should also be measured since some substances may affect other measurement variables much more than frond numbers. However, in the second available study performed with Lemna, frond number and dry weight were measured, and the frond number was the most sensitive endpoint. Therefore, it might be considered that the lack of another variable is not invalidating this study, even though the test substances are not the same in both studies. As a conclusion, DS was proposing to use this study and obtained ErC50 value of 17.45 mg a.s./L (based on geometric mean measured concentrations) for classification purpose as this value falls within the dose range where reliable analytical data is available.

With the second toxicity study on duckweed, the effect of BAS 083 34 W (mepiquat chloride 51.6 g/L, pinkish liquid) on the growth of Lemna gibba was determined in a static test over a 7-day exposure period. Test was conducted according to OECD TG 221 and EC No 761/2009, C26 (2009) guidelines and in compliance with GLP. Test item BAS 083 34 W is noted to contain only one co-formulant in very low concentration which do not have harmonised or self-classification as hazardous to the aquatic environment. The rest of the test item is water (The detailed content was provided in the confidential annex). In the test duckweed was exposed to seven concentrations of BAS 083 34 W with a control run in parallel. There were 3 replicates per test group containing 12 fronds (4 fronds from 3 plants). Test vessels were covered and incubated under controlled environmental conditions under continuous light. The tested concentration range was 1.0, 3.2, 10, 32, 100, 316, and 1000 mg test item/L. Analyses confirmed test item concentrations were in a range of 105 – 115% of nominal at the start of the test, and < LOQ (limit of quantification of 0.026 mg a.s./L) – 84% at the end. Frond production and appearance were recorded on days 2, 5, and 7. Dry weight was measured on day 0 (from a sample of similar fronds) and at day 7 in all samples after drying at 60 °C to a constant weight. Growth in controls met the validity criteria of doubling time < 2.5 days with 1.6 days. Both frond number and dry weight were assessed in this test and frond number was the most sensitive endpoint. Test concentrations of the test item dropped during the test, but the results are based on geometric mean measured concentrations. The EC50 (7-d, frond number) based on growth rate and calculated as active substance mepiquat chloride was 31.77 mg a.s./L. The study is considered valid for the classification purpose by the DS.

Two long-term studies with fish were presented in the CLH report; one 28-days sublethal test and one fish early life stage test, both conducted with rainbow trout. The DS reported that the sublethal effects of mepiquat chloride were studied to rainbow trout in 28-days test which was performed according to OECD TG 204 and following GLP. It is noted that following the OECD Council decision, the 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014. No significant deviations from the OECD TG 204 were identified. The validity criteria of the guideline were fulfilled (mortality in the control < 10%, DO > 60%, test concentrations >80% of the nominal throughout the study). Test concentrations were sufficiently maintained in the test solution (95.8 and 112.8%). The resulting endpoint of 28 d-NOEC was 100 mg/ a.s./L based on nominal concentrations. **However, the test is not considered by the DS adequate for the chronic classification, therefore, it is used only as a supportive information**.

In the early life stage test rainbow trout embryos were exposed to six mepiquat chloride (purity 598.1 g/L [measured]) concentrations and control for 95 days under flow through conditions. The endpoints evaluated were embryo hatching, the percentage of alive embryo

produced, normal larvae at hatching, larval survival and larval growth. The study was conducted according to OECD TG 210 and in compliance with GLP. No significant deviations from the test guideline were identified. The validity criteria were fulfilled; the test concentrations were sufficiently maintained throughout the study (mean concentrations were 1.01, 6.87, 13.73, 28.42, 57.36 and 110.73 mg/L corresponding to 101-114.7% of nominal), dissolved oxygen was > 60% of saturations, and test temperature maintained generally at 10 C°. Hatchability in controls was > 75%. No adverse effects were reported throughout the trial, and the NOEC was therefore 100 mg a.s./L, based on nominal concentrations.

The DS reported only one available chronic toxicity test with *Daphnia magna*. The chronic toxicity of mepiquat chloride (purity 99%) to water flea was determined in a 21-day static-renewal test. Mepiquat chloride was tested at nominal concentrations of 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L. The study was conducted according to EEC guideline XI/681/86 (equivalent to OECD TG 211) and following GLP. No significant deviations from the OECD TG 211 was apparent. The validity criteria of the guideline were fulfilled (at the end of the study in controls the mortality of parents did not exceed 20%, mean number of living offspring 7 parent > 60). The test concentrations were sufficiently maintained during the test (92.7 and 102.6% of nominal). Temperature during the study was 19.6 – 21.0 °C, pH 7.6-8.4 and dissolved oxygen 7.3 – 9.5 mg/L. Observation on the survival and reproduction were made daily. Adult survival was affected from 25 mg a.s./L with none surviving to reproduce at above concentrations. There were no adverse effects reported at concentrations below 25 mg a.s./L. **The NOEC for *Daphnia magna* was 12.5 mg a.s./L**, based on nominal concentrations and the LOEC was determined to be 25 mg a.s./L.

The DS reported that three algae studies and two studies with aquatic plant *Lemna gibba* were available. These studies were the ones reported for the acute aquatic toxicity section (cf this section for the description of these tests). The effect of mepiquat chloride (purity 99%) on the growth of green alga *Pseudokirchneriella subcapitata* was determined over a 72-hour exposure period. This study is already summarized in the acute aquatic toxicity section. Concentrations of the test item were confirmed by analysis of treated growth medium at the start and after 72-hours (concentrations tested were 10, 150 and 1000 mg a.s./L) and found to be between 103.3 and 106.0% of nominal. Therefore, the results were based on nominal concentrations. **The NOEC for biomass and growth rate was 1000 mg a.s./L.** In the second study, growth inhibition in the form of biomass and growth rate was assessed after 72 hours. **The $E_bC_{10}$ for biomass and the $E_rC_{10}$ for growth rate were 429 mg a.s./L and >1000 mg a.s./L, respectively**. Based on the growth rate and biomass, the NOEC and LOEC were 316 and 1000 mg a.s./L, respectively. Morphological effects on algae were assessed but not observed.

The study regarding the effect of BAS 083 52 W (mepiquat chloride 617.6 g/L, water based liquid formulation) on the growth of the duckweed *Lemna gibba (*study already described in the acute aquatic toxicity section) was determined over a 7 day exposure period (nominal concentrations were 0.001, 0.01, 0.1, 1.0, 10.0 and 100 mg a.s./L). In the test report the results were based on nominal concentrations, the NOEC for growth rate was determined to be 0.01 mg a.s./L and $E_rC_{10}$ was 0.15 mg a.s./L (95% confidence interval 0.12 – 0.2). Because unstable exposure conditions were observed at low mepiquat concentration (1.0 mg a.s./L), the results were also calculated based on geometric means of the concentrations in the three highest exposure concentrations (nominal 1, 10 and 100 mg/L; measured geom. mean: 0.57; 10.25 and 100.94 mg/L) resulting; $EC_{20}$ of 2.341 and $EC_{10}$

of 0.819 mg a.s./L. The DS noted that statistically significant effects were observed already at the nominal concentration of 0.10 mg/L where no measurements were available. The NOEC of 0.01 mg a.s./L and $E_rC_{10}$ of 0.15 mg a.s./L (based on nominal concentrations) are then related with some uncertainty, as actual effect concentrations might be even lower. Both NOEC and $E_rC_{10}$ values are available, and in that case $E_rC_{10}$ value is usually preferred over NOEC. $E_rC_{10}$ value of 0.819 mg a.s./L based on geometric mean measured concentrations is in same order of magnitude as $E_rC_{10}$ value of 0.15 mg a.s./L based on nominal concentrations. However, the DS noticed that there remains some uncertainty that the effect concentration might be even lower as analyses from three concentrations revealed that the mepiquat chloride dissipates at low concentrations to a large extent (1.0 mg a.s./L), but at higher concentrations it seems to be more stable. As a conclusion, the dossier submitter was proposing to use **this study as a supportive study for chronic classification of mepiquat chloride as no definitive conclusion of reliable effect concentrations for chronic hazard can be drawn**. The other Lemna study confirmed the high chronic toxicity of mepiquat chloride to aquatic plant *Lemna gibba*. The concentrations of the test item dropped during the test, but the results are based on geometric mean measured concentrations and calculated as active substance mepiquat chloride. **The NOEC was determined to be 0.03 mg a.s. /L and $E_rC_{10}$ 0.73 mg a.s./L (7-d, frond number, growth rate).** This study is considered by the DS as valid for classification purpose.

In an OECD TG 301A study, mepiquat chloride was considered readily biodegradable, as the pass level criteria of the ready biodegradation test (70% of DOC removal) was reached in a 10-day window within a 28 days period. This GLP-compliant test was performed over 35 days with the end of the lag phase, corresponding to the time when the degradation rate exceeded 10%, was reached in 19 days.

The inoculum used in this test was non preadapted and came from activated sludge of wastewater plants treating municipal sewage. Regarding the lag phase, the delay could either have been due to microbial adaptation, or to intermediate metabolites still being dissolved carbon.The reference substance, aniline, reached the pass level by day 14 indicating that the inoculum activity is under the accepted level and effective. Different appropriate controls were used during the test: blank, inhibition, abiotic and adsorption. Since mepiquat chloride is a surface active substance, the adsorption control provides particularly interesting data. Unfortunately, this control was run only during the first five days of the experiment, assuming that adsorption to the test vessel or sludge would likely occur in the early days of the experiment. Thus, during the studies test substance replicates 1 and 2 showed a significant DOC decrease in the test substance measured from 24 days onwards. In addition, the abiotic flasks (containing only test substance and aquatic medium) showed no decrease in DOC over the 28-day period, confirming no adsorption to the walls of the vessel over the extended period. Furthermore, in the inhibitory control flask, the first plateau is linked to the end of aniline degradation. If mepiquat chloride adsorption occurred late in the test, a DOC decrease would be observed during this step. Since such a decrease was not observed, RAC considers this study as valid, indicating that according to the CLP criteria mepiquat chloride should be considered readily biodegradable for classification purposes.

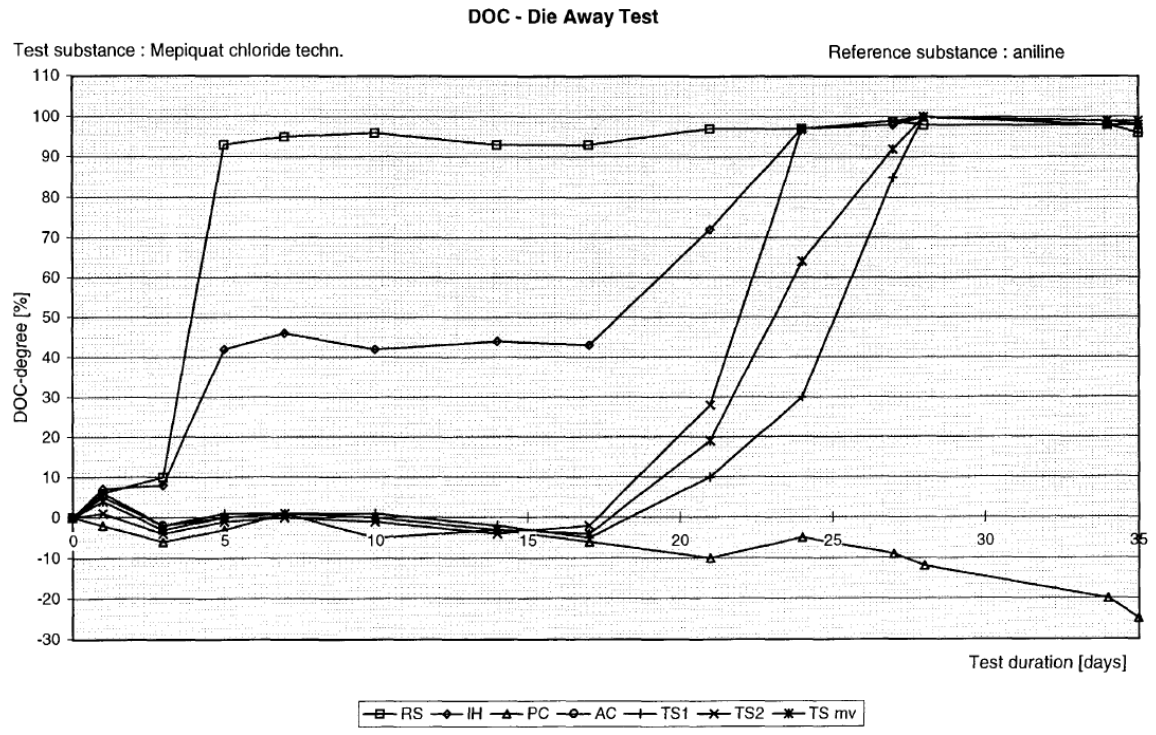



Figure: Degradation curves on mepiquat chloride study (RS : Reference substance (Aniline), IH: Inhibition control, PC: abiotic control, AC: adsorption control, TS1: test substance 1, TS2: test substance 2, TS mv: test substance mean value

## 12 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

## 13 ADDITIONAL LABELLING

## 14 REFERENCES

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## 15 ANNEXES

Finland, 2020. draft Renewal Assessment Report (dRAR) on mepiquat chloride prepared by the rapporteur MemberState Finland in the framework of Commission Implementing Regulation (EU) No 844/2012, January 2020.