Committee for Risk Assessment

RAC

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

Adopted

18 March 2021

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18 March 2021

CLH-O-0000006959-53-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride**

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

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

The proposal was submitted by **Finland** and received by RAC on **27 February 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Finland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **31 March 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 June 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Michal Martínek**

Co-Rapporteur, appointed by RAC: **Laure Geoffroy**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	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	mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride	246-147-6	24307-26-4	Acute Tox. 4* Aquatic Chronic 3	H302 H412	GHS07 Wng	H302 H412	-	-	-
Dossier submitters proposal	613-127-00-7	mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride	246-147-6	24307-26-4	**Modify** Acute Tox. 3 **Add** Acute Tox. 4 STOT SE 2 Repr. 2 **Retain** Aquatic Chronic 3	**Modify** H301 **Add** H332 H371 (nervous system) H361d **Retain** H412	**Modify** GHS06 Dgr **Add** GHS08	**Modify** H301 **Add** H332 H371 (nervous system) H361d **Retain** H412	**-**	**Add** oral ATE = 115 mg/kg bw inhalation ATE = 2.8 mg/L (dusts or mists)	**-**
RAC opinion	613-127-00-7	mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride	246-147-6	24307-26-4	Acute Tox. 3 Acute Tox. 4 Aquatic Chronic 3	H301 H332 H412	GHS06 Dgr	H301 H332 H412		oral: ATE = 270 mg/kg bw inhalation: ATE = 2.8 mg/L (dusts or mists)	
Resulting Annex VI entry if agreed by COM	613-127-00-7	mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride	246-147-6	24307-26-4	Acute Tox. 3 Acute Tox. 4 Aquatic Chronic 3	H301 H332 H412	GHS06 Dgr	H301 H332 H412		oral: ATE = 270 mg/kg bw inhalation: ATE = 2.8 mg/L (dusts or mists)	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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 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) 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 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)**.

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.

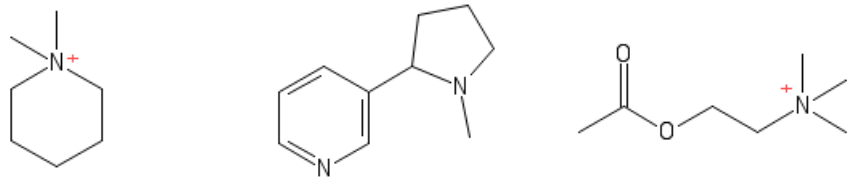
Findings potentially relevant for a STOT SE classification			
Study	**Dose (concentration), non-lethal**	**Clinical signs at non-lethal dose**	**Other findings;** **Symptoms potentially related to neurotoxicity at lethal doses**
Oral gavage			
Acute toxicity, rat (1989)	120 mg/kg bw	None	270 mg/kg bw: mortality (5/10), staggering, twitching, compulsive gnawing
Acute neurotoxicity, rat (2002)	170 mg/kg bw	None	FOB: reduced motor activity (males, day 0) 700 mg/kg bw: mortality (1/20), lethargy, abdominal position, unsteady gait,

Findings potentially relevant for a STOT SE classification			
Study	**Dose (concentration), non-lethal**	**Clinical signs at non-lethal dose**	**Other findings;** **Symptoms potentially related to neurotoxicity at lethal doses**
			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 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 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 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 Week 2: tremor (7/20) Week 4: tremor (18/20) From week 5: tremor, abnormal gait (long-legged, unsteady), abdominal position	FOB week 5: tremors, ataxia, posture abnormality, reduced grip strength Food consumption: week 1 reduced by ca. 60%, week 5 by ca. 30%/20% m/f 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 Histopathology: axonal degeneration tibial nerve males 2/5, grade 1 (not

Findings potentially relevant for a STOT SE classification			
Study	**Dose (concentration), non-lethal**	**Clinical signs at non-lethal dose**	**Other findings;** **Symptoms potentially related to neurotoxicity at lethal doses**
			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 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 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	The treatment started with a higher dose (8000 ppm), but due to mortalities (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 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**.

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.

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.**

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.

Developmental neurotoxicity study in rats (2006)

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**.

ENVIRONMENTAL HAZARD EVALUATION

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

A 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 warrant classification as Acute Aquatic toxicity**.

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.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).