Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Adopted

18 March 2021

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: mepiquat chloride (ISO); 1,1-dimethylpiperidinium chloride
EC number: 246-147-6
CAS number: 24307-26-4
Dossier submitter: Finland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	1
Comment received				
FR: Composition of the substance (Page 4): According to the RAR (January 2020),the purity of the substance is 990 g/kg (dry technical material, TC) and 615-665 g/L (technical concentrate, TK). Please could you report this information FR: Composition of the substance (Page 5): According to the LOEP of RAR (January 2020), the content of N-methylpiperidine is max. 3 g/kg (TC).				
Dossier Submitter's Response				
Thank you your comment. We will add this information to the report.				
RAC's response				
Thank you, noted.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	2
Comment received				
DE-CA agrees with Acute Tox 3 (H301), Acute Tox 4 (H332), STOT SE 2 (H371, nervous system), and with Repr.2 (H361d). Chapter 7 „Physicochemical properties", Table 8: in the column „Reference" the year of the study and the author should be added. Additionally, the exact dRAR source should be named.				

Dossier Submitter's Response
Thank you for your support. We have used references to the dRAR document. Physichemical properties are in the Volume 3, Annex B.2: Physical and chemical properties of the active substance. Exact dRAR sources/references are already in the table 8.
RAC's response
Thank you, noted.

Date	Country	Organisation	Type of Organisation	Comment number
14.04.2020	United Kingdom	IPI Global Ltd	Company-Manufacturer	3
Comment received				
CLOSED LOOP SYSTEM AS A TECHNICAL ALTERNATIVE PROCESS OR PROCEDURE FOR SAFELY HANDLING MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE. It is very well recognized that closed loop system technology reduces the exposure of the operator below the threshold recommended by the EU as confirmed by our customers and the HSE study and that it is in agreement with the latest amended EU directives for CMD 2004 and in general with the (89/391/EEC) of 12 June 1989 and the 89/24/EC of 7 April 1998. ECHA note – An attachment was submitted with the comment above. Refer to public attachment MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE.pdf				
Dossier Submitter's Response				
We acknowledge your comment.				
RAC's response				
Thank you for the comment. However, hazard classification is based on intrinsic properties of the substance. Exposure considerations are part of risk assessment.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	4
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	5
Comment received				
The DS formerly proposed to classify the substance as Carc. 2, H351 (EU renewal of approval procedure). After the commenting round, the Rapporteur Member State (RMS) combined the findings from the chronic toxicity and carcinogenicity part of the rat study				

(Mellert and Hildebrandt 1994) and decided not to propose classification.
We agree that the CLP criteria for classification are not fully met. However, some uncertainties remain considering the increased incidences of brain tumours in both sexes:

• males: meningioma 0-0-0-1 (1.4 %) at 0-290-2316-5790 ppm corresponding to 0-13/17-105/141-269/370 mg/kg bw/d for m/f, incidence is outside of HCD (mean 0.49 %) and schwannoma: 0-0-0-1 (1.4 %) at 0-290-2316-5790 ppm, outside of HCD (mean 0.1%)
• females: glioblastoma 0-0-0-2 (2.9 %) at 0-290-2316-5790 ppm (HCD: 0.69 % if glioma, glioblastoma and oligodendroglioma are combined and 0 % for glioblastoma).

In the opinion of DE, the increased incidence of this rare tumour type seems to be a borderline case. Another uncertainty lies in the fact that some tumour types (urothelial papilloma in males, adenocarcinoma of mammary gland in females, stromal polyp in uterus and uterus adenocarcinoma) occur within the range of HCD but above the mean. But overall, it is considered to be not sufficient to propose Carc. 2 H351, in particular because of the confusing change of nomenclature of brain tumours, which has to be taken into account.

Dossier Submitter's Response

We acknowledge your comment. Thank you for your support.

RAC's response

Thank you for you comment. RAC agrees that the few differences in tumour incidence between the control and high dose group in the 2-year rat study (1994) that were without statistical significance and more or less within the HCD range (e.g. uterine adenocarcinoma) are not sufficient for classification.
As to meningioma, it is related to granular cell tumours. Combined incidence of meningiomas and granular cell tumours is 5/70 in control males and 2/70 in top dose males according to the original study reports.
Similarly, when combining gliomas (i.e. oligodendroma, glioblastoma, undifferentiated glioma) for females, the incidence is 2/70 in control females and 2/70 in top dose females.

Date	Country	Organisation	Type of Organisation	Comment number
29.05.2020	Germany	BASF SE	Company-Manufacturer	6

Comment received

BASF agree with the opinion of the Dossier Submitter (DS) that Mepiquat-chloride is not carcinogenic in the rat or mouse and therefore does not meet the criteria for classification for carcinogenicity:
- In both the 24 month chronic toxicity study and 24 month carcinogenicity study, there were no treatment-related increases in tumour incidence either individually or overall
- There is no evidence of any pre-neoplastic lesions that suggest the potential for a tumour outcome
- The observed tumour incidence is within the spontaneous background range of historical control data from the test laboratory
- There are no indications of genotoxicity

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mepiquat Response to Public Consultation for CLP 28 May 2020_Attachment 2020_2081825.pdf
Dossier Submitter's Response
Thank you for your comment.
RAC's response
Thank you for your comment. RAC agrees that classification for carcinogenicity is not warranted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	7
Comment received				
We support the proposal to classify the substance as Repr. 2, H316d. In the rabbit developmental toxicity study (Bachmann et al. 1998), two pups (2 %) revealed hydrocephalus at the high dose of 150 mg/kg bw/d. As confirmed by RMS in the PREV meeting 25, the two foetuses were from the same litter. However, the incidence is outside of HCD (one case of hydrocephalus out of 2459 fetuses examined ☐ 0.08%). Furthermore, hydrocephalus was also observed in the rat developmental study and in the 2-generation study in rats. The incidence of observed hydrocephalus in foetuses (n=2) at the highest dose level, which were seen in the developmental toxicity study in rats (Hellwig and Hildebrandt 1992) is not higher than in the control group (n=2). However, it is observed above HCD. The slight increase in hydrocephalus in 2 species in 3 studies is considered sufficient to justify classification as Repr. 2, H361d.				
Dossier Submitter's Response				
We acknowledge your comment. Thank you for your support.				
RAC's response				
Thank you for your comment. A slight increase in hydrocephaly was observed in the rabbit PNDT study, with foetal (litter) incidence of 0, 1, 0, 2(1). 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 are related to treatment. RAC notes that the two affected top-dose foetuses 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. In general, concurrent control is the primary reference. Therefore RAC does not share your view that the non-existent increase in the incidence of hydrocephaly in the rat PNDT study (2 foetuses from 2 litters in the control, 2 cases from 2 litters in the top dose group) supports classification. As to the 2-generation study, there was a single F1b pup with hydrocephaly the mid-dose. No case of hydrocephaly was found at the top dose in F1b nor in any treatment group of F1a or F2. Therefore, the single case of hydrocephaly in the 2-generation study is unlikely to be related to treatment. For discussion of other effects see the opinion.				

Overall, RAC is of the view that classification of mepiquat chloride for developmental toxicity is not justified.

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	8
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.05.2020	Denmark		MemberState	9
Comment received				
DK supports the classification for Repr 2; H361d (suspected of damaging the unborn child) suggested by the DS, based on the findings of hydrocephalus and anophtalmia in several studies. Although hydrocephalus was observed in the control group in one study, this was not the case for two other studies, and since the HCD show that both hydrocephalus and anophtalmia is rarely seen, the effects were considered treatment related.				
Dossier Submitter's Response				
Acknowledged. Thank you for your support.				
RAC's response				
Thank you for your comment. Hydrocephalus is discussed in the response to comment 7. A single pup with unilateral anophthalmia was observed in the 2-generation study. It was the same F1b pup that also had hydrocephaly. Again, there was no other case of anophthalmia in top dose F1b pups nor in any F1a or F2 pups. Several cases of anophthalmia are found in historical controls (5 cases in 3 litters out of 13 studies, 1987-1992). A single case of unilateral anophthalmia also occurred at the mid-dose in the rat PNDT study (1992). No anophthalmia was observed in the concurrent control nor in historical controls for the PNDT study but several cases occured in the HCD for the 2-generation study discussed above (the same laboratory and source of animals). This single case without a dose-response relationship is likely to be a chance finding and is not considered to support classification. For discussion of other effects see the opinion. Overall, RAC is of the view that classification of mepiquat chloride for developmental toxicity is not justified.				

Date	Country	Organisation	Type of Organisation	Comment number
29.05.2020	Sweden		MemberState	10

Comment received

From the data presented in the CLH-report, the Swedish CA is of the opinion that the classification for mepiquat chloride as Repr. 2 (H361d) cannot be primarily/solely based upon anophthalmia and hydrocephaly as foetal malformations, as concluded by the DS.

In the two-generation study in rats (Study Report, 1993), hydrocephaly and anophthalmia were seen only in 1 pup (not specified if the same pup) from the mid-dose group. In the PNDT study in rats (Study Report, 1992) anophthalmia was reported in 1/297 foetuses (no HCD available), whereas hydrocephaly was seen in 1/150 and 2/151 foetuses at 50 and 300 mg/kg bw, respectively, but also in 2/129 foetuses in the control group. The incidence of hydrocephaly seen in the controls is higher than the provided HCD range (dating 1991-1995), i.e. 1/3091 foetuses. In the PNDT study performed in rabbits (Study Report, 1998), hydrocephaly was observed in 1/96 and 2/102 foetuses at 50 and 150 mg/kg bw, respectively, and in 0/92 foetuses in the control group (HCD: 1/2459 foetuses).

The incidences of hydrocephaly and anophthalmia do not show a dose-response relationship, are very low and not stat. sign. different from controls and/or not outside the HCD range and thus, these data are not considered as some evidence and do not warrant classification of mepiquat chloride as Repr. 2.

In addition, we have some concerns regarding the purity of the test substance in all three studies mentioned above. It appears as the purity of 57.9% stated in the summaries of the studies in the CLH-report is the amount of mepiquat chloride used to obtain an aqueous dilution for the use as a plant growth regulator. Consequently, the purity of the test substance seems to be unknown. On the other hand, if indeed 57.9% is the correct degree of purity, then we strongly doubt that the available studies are reliable since the question arises: what is tested then?

Dossier Submitter's Response

Thank you for your comments. We agree that the hydrocephalus and microphatlmia finding do not show a dose-reponse relationship. However, as they are a rather rare occurance in rats or rabbits, taken altogether as weight of evidence of all studies there seemed to be support for classifying in category 2.

RAC's response

Thank you for your comments. RAC agrees that there is no convincing evidence of a treatment-related increase in hydrocephalus and microphthalmia in the available studies with mepiquat chloride.
As to purity of the test substance, RAC confirms, based on the original study reports, that the substance tested in the key studies (2-generation study 1993, rat PNDT study 1992, rabbit PNDT study 1998) was a technical concentrate, an aqueous solution containing ca. 57-58% of mepiquat chloride, and that the dose levels in these studies as provided in the CLH report are after correction for purity, i.e. they correspond to pure mepiquat chloride.

Date	Country	Organisation	Type of Organisation	Comment number
29.05.2020	Germany	BASF SE	Company-Manufacturer	11

Comment received

Proposal for H361d (Suspected of damaging the unborn child): No Classification is Required as the CLP Criteria are Not Met for Developmental Toxicity

In a comparison with the CLP criteria, the CLH report states the following (p. 75): "There is evidence from reliable developmental toxicity studies in two species that mepiquat causes fetal malformations, namely, hydrocephalus and anophthalmia. 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."

The CLP criteria for Repr. 2, H361d are not met. There is overwhelming evidence that mepiquat-chloride does not cause hydrocephalus or anophthalmia in the rat or rabbit. The incidence of these findings clearly follows a pattern that is consistent with isolated spontaneous occurrence with no relationship to treatment. The findings of the developmental and reproductive toxicity studies are reviewed here in detail with regard to the weight of evidence supporting a lack of any specific effects on development.

Two-Generation Reproductive Toxicity Study in Rats BASF Doc ID 1993/10983

The CLH report cites the observation of isolated incidences of anophthalmia and hydrocephaly in the two-generation reproductive toxicity study (in-life phase of study conducted in 1991) as being supportive of their proposal to classify with H361d (p. 74). As acknowledged by the Dossier submitter (DS), the single incidences of anophthalmia and hydrocephaly both occurred in the same pup at the mid-dose and was only seen in the F1b litter but not the F1a or F2 litters. There were no other incidences of these malformations in any other dose group. Although not stated by the DS in the CLH report, the incidence of both findings was within the range of historical control data (HCD) for the test laboratory which cover the period from 1987 to 1992. The incidence of hydrocephaly at the mid-dose of 1500 ppm was 1/25 litters (4.0%) and 1/364 fetuses (0.3%) compared to a historical control range of 0-4.2% with a mean of 0.1% (1/754) for litter incidence and 0-0.3% with a mean of 0.01% (1/9647) for fetal incidence. The incidence of anophthalmia at 1500 ppm was 1/25 litters (4.0%) and 1/364 fetuses (0.3%) with a historical control range of 0-4.5% with a mean of 0.4% (3/754) litter incidence and 0-1.1% with a mean of 0.05% (5/9647) for fetal incidence. Refer to Table 1 in the attachment for further details.

These two malformations (single incidence of hydrocephaly and a single incidence of anophthalmia) co-occurred in the same pup indicating that, in all probability, they were genetic in origin and unrelated to treatment. This spontaneous observation provides no supporting weight of evidence for classification with H361d.

In addition to the spontaneous finding outlined above, other offspring findings the CLH report noted (p. 74) in the high dose group of the two generation reproductive toxicity

were the reduction in mean body weight gain of high dose F1a, F1b and F2 pups; a lower number of F1a, F1b and F2 pups reaching the criteria of auditory canal opening and eye opening and gripping reflex; an increased number of F1a pups died and were cannibalised and F1 pups had lower viability and lactation indices. These findings are considered to be secondary non-specific consequences of the marked maternal toxicity that was observed at the top dose in this study.

The maternal toxicity observed at the high dose was most marked during lactation when severe clinical signs of toxicity, including tremors and hypersensitivity, and insufficient or no nesting activity were observed. During lactation, at 5000 ppm 17/20 F0 dams of the F1a litter, 15/23 F0 dams of the F1b litter and 15/20 F1 dams of the F2 litter exhibited tremors and/or hypersensitivity, the onset of which was coincident with reduced food consumption and body weight gain. Maternal food consumption at the top dose was decreased markedly during lactation of the F1a, F1b and F2 litters, (by 23, 21 and 21%, respectively) resulting in maternal body weight losses (0.2, 2.0 and 3.3 g losses compared to gains of 21.0, 10.7 and 7.9 g in controls for the F1a, F1b and F2 litters respectively) during this critical phase of pup development. This is reflected by the fact that Day 1 pup weights at 5000 ppm were only marginally lower than controls with the effect on pup weight becoming much more pronounced (22-28%) by Day 7, prior to any consumption of test diet by the pups, indicating that the observed maternal toxicity during lactation had a direct secondary non-specific consequence upon pup growth and development. There were no effects on maternal body weight or clinical condition in the mid and low dose groups and consequently there were no effects on pup weight or development indicating that the effects at the high dose are a secondary non-specific consequence of maternal toxicity. Refer to Tables 2 and 3 in the attachment for further details.

The were some slight indications of developmental delay at the top dose with statistically significant reductions in the number of pups reaching the development criteria for auditory canal opening and eye opening and gripping reflex. For pinna unfolding at 5000 ppm, the % of pups achieving this landmark was 65%, 73% and 81% for the F1a, F1b and F2 litters compared to respective control values of 94%, 93% and 93% and a historical range (studies conducted 1987 to 1992) of 74-100%. For auditory canal opening at 5000 ppm, the % of pups achieving this landmark was 67%, 72% and 92% for the F1a, F1b and F2 litters compared to respective control values of 99%, 97% and 96% and a historical range of 81-100%. For eye opening at 5000 ppm, the % of pups achieving this landmark was 99%, 73% and 81% for the F1a, F1b and F2 litters compared to respective control values of 100%, 95% and 99% and a historical range of 85-100%. For gripping reflex at 5000 ppm, the % of pups achieving this landmark was 99%, 94% and 97% for the F1a, F1b and F2 litters compared to control values of 100% for all litters and a historical range of 98-100%. For pupil constriction at 5000 ppm, the % of pups achieving this landmark was 99%, 95% and 98% for the F1a, F1b and F2 litters compared to respective control values of 99%, 100%, and 100% for the F1a, F1b and F2 litters and a historical range of 95-100%. Refer to Table 4 in the attachment for further details.

These delays in development are secondary consequences of the effects on pup weight (22-28% decrease by post-natal Day 7) caused by the marked maternal toxicity seen during lactation.

The values for lactation and viability were, with one exception (F1a litter) within the historical control range (studies conducted 1987 to 1992) and were not statistically significantly different from concurrent controls indicating that there are no treatment-related effects on these end points. Although lactation index was marginally lower than controls for the F1a litter at 5000 ppm (95% in controls compared to 91% at 5000 ppm; historical range 95-100%), this was not evident in the F1b or F2 litters. Refer to Table 5 in the attachment for further details.

The high number of pups cannibalized and higher number of pup deaths on days 1-4 at the top dose of 5000 ppm is a secondary consequence of marked maternal toxicity, especially during lactation. The lower total number of pups delivered at the top dose in the F1a and F2 litters reflects the lower total number of litters in these groups with no differences seen in these end-points in the F1b litter.

It is concluded that the offspring effects seen only at the top dose in the two generation reproductive toxicity study occur as a secondary non-specific consequence of maternal toxicity which was particularly marked during lactation as was evident by severe clinical signs of toxicity and body weight loss.

The two generation reproductive toxicity study in rats does not provide any weight of evidence for effects on development that meet the criteria for classification with H361d.
Developmental Toxicity in the Rat (BASF Doc ID 92/10331)

Female Wistar rats were dosed with mepiquat-chloride by gavage at 0 (distilled water), 50, 150 and 300 mg/kg bw/day, from days 6 to 15 (inclusive) of gestation. At the high dose there were marked clinical signs of toxicity including tremor, unsteady gait, piloerection and hypersensitivity were observed between days 6 to 14 of gestation and ataxia was seen on days 7-9 of gestation. At the top dose, body weight gain was statistically significantly decreased during gestation by around 13-16% and food consumption was also significantly reduced by 10-19% compared to controls.

The percentage litter incidence of hydrocephaly was higher in controls than in the high dose group with 2/20 (10%) litters affected in controls versus 2/23 (8.7%) at the top dose. Similarly the fetal incidence of hydrocephaly was also higher in controls than in the high dose group with 2/129 (1.6%) fetuses affected in controls versus 2/151 (1.3%) fetuses in the high dose group. Historical control data (HCD; BASF DocID 2019/2075940) from the test laboratory covering the period 1991 to 1995 (in-life phase of study was conducted in April 1991) indicate that one fetus out of 3664 (0.03%) had hydrocephaly. The CLH report inaccurately states the historical incidence to be one case out of 3901 (0.03%) fetuses (p. 62). The incidence of hydrocephaly in the high dose and the control group was higher than the historical control range. The proposal that the incidence of hydrocephaly observed in this study is related to treatment with mepiquat-chloride lacks biological plausibility as the % incidence is higher in controls than in treated animals.

A single incidence of anophthalmia was observed in the mid-dose group but not in any other treated group nor controls. Anophthalmia was not reported in the HCD (studies conducted from 1991-1995) although one case of microphthalmia was observed out of 7606 fetuses examined. The CLH report inaccurately states the historical incidence as being one out of 8105 fetuses (p. 62). The single incidence of anophthalmia in the mid-dose group represents a spontaneous finding that is unrelated to treatment with mepiquat

chloride.

The CLH report states that the total fetal and litter incidence of soft tissue variations and the fetal incidence of dilated renal pelvis were higher in the mid and high dose groups compared to controls (p. 62). However, there were no statistically significant differences from control for these observations which occur with a high spontaneous frequency as reflected by the HCD from 1991-1995. The litter incidence of total soft tissue variations was 50%, 50%, 57% and 61% in concurrent controls and the respective dose groups compared with the historical control range (studies conducted from 1991-1995) of 33.3-80% The fetal incidence of soft tissue variations was 11%, 11%, 13% and 14% in the concurrent control and respective dose groups compared to the historical control range of 4.9-33.1%. The litter incidence of dilated renal pelvis was 50%, 50%, 57% and 61% in concurrent controls and the respective dose groups compared with the historical control range of 33.3-80%. The fetal incidence of dilated renal pelvis was 11%, 11%, 13% and 14% in the concurrent control and respective dose groups compared to the historical control range of 4.9-33.1%. Refer to Table 6 in the attachment for further details.

There are no treatment-related findings in the rat developmental toxicity study (BASF Doc ID 92/10331) to indicate that mepiquat-chloride has any adverse effects on development. The study findings do not meet the CLP criteria for classification with H361d.

Developmental Toxicity in the Rabbit (BASF Doc ID 1998/10497)

Himalayan rabbits were dose with mepiquat-chloride by gavage doses of 0 (distilled water), 50, 100 and 150 mg/kg bw/day, from day 7 to day 19 of gestation; the in-life phase of the study was May-July 1997. At the high dose body weight loss was observed during days 7 - 19 of gestation with a mean body weight loss of 50 grams over the entire treatment period compared to a weight gain of 44.6 g in controls. There was also a 42% reduction in food consumption during gestation in the high dose group and two animals were observed to have blood in their bedding on Days 23-27.

The litter is the conventional unit for assessment of developmental defects since findings may be due to genetics of the dams. Hydrocephaly occurred in 1 fetus (1/92 = 1.1%) at the low dose and 2 fetuses (2/102 = 2.0%) from the same litter at the high dose; a dose causing marked body weight loss during treatment. There were no statistically significant differences between the groups for the incidence of hydrocephaly. The historical control incidence of hydrocephaly, from studies conducted during 1995-2000, (see BASF Doc ID 2019/2075941) was one case reported out of 1230 fetuses/189 litters examined (mean of 0.08% with a range of 0-0.6% for fetal incidence and a mean of 0.5% with a range of 0-4.2% for litter incidence). The CLH report inaccurately states the historical control incidence as being one case out of 2459. The absence of any hydrocephaly in the mid-dose together with the fact that the two incidences of hydrocephaly at the top dose were observed in the same litter indicates that these are spontaneous observations unrelated to treatment with mepiquat-chloride.

The litter (64%, 87%, 73% and 86% in control and treated groups) and fetal incidence (17%, 20%, 22% and 25%) of total skeletal variations is well within the spontaneous background incidence, as reflected by the historical control data (mean litter incidence of 87.8% with a range of 58.8-100% and mean fetal incidence of 48.3% with a range of

11.5-73.5%) and there were no statistically or biologically relevant differences between the concurrent control and treated animals indicating that there is no treatment-related effect. There is no justification for hazard classification based on the prenatal developmental toxicity study in rabbits. Refer to Table 7 in the attachment for further details. There are no treatment-related findings in the rabbit developmental toxicity study (BASF Doc ID 1998/10497) to indicate that mepiquat-chloride has any adverse effects on development. The study findings do not meet the CLP criteria for classification with H361d. Overall Conclusion Regarding proposal for H361d In conclusion, the CLP criteria for classification for developmental toxicity (H361d) are not met. The observation of isolated occurrences of anophthalmia and hydrocephaly cited by the DS in support of classification are clearly isolated spontaneous findings that within the historical control range and are therefore unrelated to treatment with mepiquat-chloride. The DS proposal that these observations are related to treatment has no biological plausibility. For further explanations including tabular overviews please refer to the attachment to the comments (BASF DocID 2020/2081825) ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mepiquat Response to Public Consultation for CLP 28 May 2020_Attachment 2020_2081825.pdf
Dossier Submitter's Response
Thank you for your comments. We agree that the hydrocephalus and microphtalmia do not show a dose-response. However, since they occurred in various studies and are a relatively rare occurance, it was considered that there is sufficient weight of evidence for classification in category 2 H361d. Thank you for pointing out the erroneous historical control numbers.
RAC's response
Thank you for your comments. RAC agrees that classification of mepiquat chloride for developmental toxicity is not warranted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	12
Comment received				
We support additional classification for acute inhalation toxicity 4 (H332).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you, RAC agrees that Acute Tox. 4; 332 is justified.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	13
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.05.2020	Denmark		MemberState	14
Comment received				
DK supports classification for acute oral and inhalation toxicity as well as the ATE proposed for both categories.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you, RAC agrees with Acute Tox. 3 for the oral route and Acute Tox. 4 for inhalation. For the oral route RAC proposes an ATE of 270 mg/kg bw, a value considered to represent an LD_{50} from the key rat study (1989).				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	15
Comment received				
We would like to point out, that in the newly submitted LLNA study (page 19), the test substance was solved in ethanol/water (3+7 v/v). In OECD TG 429/2010, it is stated that "particular 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 L 92). Thus, wholly aqueous vehicles are to be avoided." We politely suggest to incorporate a rationale for using ethanol/water as vehicle system. However, we agree that the study does not show skin sensitisation potential of the substance.				
Dossier Submitter's Response				
Thank you for your comment. We acknowledge that using aqueous vehicles shoud be avoided and the vehicle should have been selected more carefully. However, our view is that the study is accebtable and the choosen vehicle has not most likely affected to the results.				
RAC's response				
Thank you for your comment. As ethanol has good wetting properties and mepiquat chloride possesses surface activity, RAC does not have a concern about wetting properties of the test solutions.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	16
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	17
Comment received				
We support the proposed classification STOT SE 2, H371 based on effects on the nervous system observed in the acute oral neurotoxicity study in rats, 3 month study in dogs and in the DNT study in rats.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment. RAC is of the view that clinical signs of neurotoxicity at doses causing mortality are already covered by the acute toxicity classification. Significant neurotoxicity occurred also at non-lethal doses. In the rat PNDT study tremor and hypersensitivity without concurrent mortality were observed at 300 mg/kg bw/d. As this dose level 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. Slight sedation in the 3-month dog study (1977) would be consistent with a STOT SE 3 classification for narcotic effects. However, it was not reproduced in the more recent dog studies (1994) using higher doses. Due to this lack of consistency between studies, a STOT SE 3 classification is not considered warranted either.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	18
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.05.2020	Denmark		MemberState	19
Comment received				
DK supports classification for STOT-SE in category 2 since there is evidence of neurotoxic effects in several studies also at non-lethal doses that fall within the dose-range suggested for this category. Summaries of the studies in dogs that are mentioned in the text could be added to to table 62.				
Dossier Submitter's Response				
Thank you for your support. Indeed, summaries of the dog studies could have been included in the Table 62. Further information on these studies is available in dRAR.				
RAC's response				
Thank you for your comment. Please see the response to comment 17.				

Date	Country	Organisation	Type of Organisation	Comment number
29.05.2020	Germany	BASF SE	Company-Manufacturer	20
Comment received				
Specific Target Organ Toxicity after a Single Exposure; the CLP Criteria are Not Met Classification with STOT-SE 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. The Dossier Submitter (DS) focused on what they consider to be evidence of neurotoxicity across a range of studies, including effects seen after repeat dosing, as the basis for STOT-SE2. However, the findings that the DS state in their justification (P. 87) for STOT-SE2 occur at doses causing mortality and are already addressed through classification for acute toxicity. Guidance on the application of the CLP Criteria states (3.8.2.5): "care should be taken not to assign each class for the same effect, in other words a double classification for the same effect has to be avoided". There is no evidence of specific target organ toxicity to the peripheral or central nervous system. The four studies cited by the DS in support of STOT-SE2 are the acute neurotoxicity study in the rat, acute oral toxicity (LD50) studies in the rat and mouse and the acute inhalation study in the rat. In the rat acute neurotoxicity study (BASF Doc ID 2002/1004002), there was significant toxicity at the top dose of 697 mg/kg bw as indicated by the death of 1 male on the day of administration and a 22% decrease in body weight gain by Day 7 post exposure in top dose males. There were a range of signs of toxicity observed in the top dose either prior to or during the FOB tests (abdominal position, slight/half closure of eyelids, piloerection, squatting and abdominal posture, gasping/respiratory sounds, slight or moderate tremors, impairment of coordination and unsteady or shuffling gate) and a range of effects on functional activity, rearing and motor activity which are all considered to reflect generalised toxicity (i.e. reduced activity of rats experiencing peri-lethal toxicity and				

feeling unwell) and are not specific indications of neurotoxicity. There were no effects on neuropathology. Although there was a slight decrease in motor activity in males at the mid dose of 174 mg/kg bw/d which was statistically significant for intervals 1 and 2 , this was not seen in females, was considered not to be toxicologically adverse and, in all likelihood, is unrelated to treatment. The statistically significant differences in rearing and grip strength observed in the mid-dose are unrelated to treatment with mepiquat-chloride. Refer to Tables 8 to 11 in the attachment for further details.

Overall the acute neurotoxicity study provides no specific indications of a neurotoxic effect at non-lethal doses and none of the study findings meet the criteria for classification with STOT-SE.

In the acute oral toxicity study in rats (BASF Doc ID 89/0462), mortality (2/5 males and 3/5 females) occurred at doses of 270 mg/kg bw of mepiquat-chloride and above with no signs of toxicity observed at doses below this (58 & 115 mg/kg bw). Thus, the clinical signs of toxicity (poor general state, dyspnea, apathy, abdominal position, staggering, twitching, compulsory gnawing and cyanosis) that the DS noted at 270 mg/kg bw and above only occurred at doses causing lethality. Similarly in the mouse acute oral toxicity study (BASF Doc ID 89/0463), clinical signs of toxicity were only observed at doses causing mortality (270 mg/kg bw and above). Refer to Table 12 in the attachment for further details.
Therefore the acute oral toxicity study findings in the rat and mouse do not meet the criteria for classification with STOT-SE. The mortality and associated clinical signs of toxicity in this study are addressed by the proposed Acute Tox. 3, H301 classification.

In the rat acute inhalation toxicity study (BASF Doc ID 91/10505), the clinical signs indicative of toxicity (poor general state, dyspnea, apathy, abdominal position, staggering, twitching, compulsory gnawing and cyanosis) were only observed at a concentration causing mortality (i.e. 2.84 mg/l). At the lower concentration, (1.50 mg/l) the only clinical signs observed were those associated with the experimental procedure which were irregular, accelerated and intermittent respiration and eyelid closure seen in all animals during exposure and ruffled fur, accelerated and intermittent respiration were seen in the first day post exposure. These are common findings very frequently recorded in acute inhalation studies which, like other acute toxicity studies, do not have a control group and involve restraint of the animals within restraining tubes in order to allow for nose-only exposure. Refer to Table 13 in the attachment for further details.

None of the findings in this study meet the criteria for STOT-SE2. The mortality and associated clinical signs of toxicity in this study are addressed by the proposed Acute Tox. 4, H332 classification.

In addition to the studies above, the DS also cite the following studies as providing information relevant to their STOT-SE2 proposal (p. 80-86):

In the rat sub-chronic neurotoxicity study (BASF Doc ID 2002/1011417), rats were administered dietary dose levels up to 13,000 ppm (517 and 617 mg/kg bw/d in males and females respectively). The decreases in body weight gain in top dose males and females were marked, particularly during the first month of the study indicating a marked degree of systemic toxicity in these animals. After two weeks of dosing, body weight gain in males at the top dose was 46% lower than controls and in females weight gain was

57% lower. Weight gain continued to be significantly decreased throughout the study and was 28-29% lower than controls in both sexes at the end of the study. Refer to Table 14 in the attachment for further details.

Group mean body weight and food utilization were also decreased at the top dose. Consequently, the occasional statistically significant decreases in grip strength and systemic toxicity are attributable to marked systemic toxicity. There were no statistically significant differences in motor activity.

Similar reductions in grip strength and clinical signs of toxicity were seen in the 90 day rat study conducted at a doses of 12,000 ppm (826/951 mg/kg bw/d) which caused a 32% reduction in mean body weight in males and 17% reduction in females. The moderate multifocal muscle fibre degeneration with reactive myositis observed in the gastrocnemius muscle of a single top dose male, the single incidence of axonal degeneration in sciatic nerve and two incidences in the proximal tibial nerve were considered to be spontaneous findings unrelated to treatment.

There were no effects on the functional observational battery, hindlimb grip strength or histopathology of neurological tissue in a 24 month chronic toxicity study in rats up to doses of 826-951 mg/kg bw/d in males and females respectively.

Neither the sub-chronic neurotoxicity study nor 3 month sub-chronic toxicity study in rats provide any evidence of specific target organs toxicity even after multiple doses. The observed effects only occur at doses causing significant systemic toxicity and therefore reflect non-specific secondary consequences of such repeated-dose toxicity. The findings of these repeat dose studies are not applicable to the criteria for STOT-SE nor do they provide evidence of neurotoxicity.

In the 2-Generation reproductive toxicity study in rats (BASF Doc ID 93/10983), the top dose of 5000 ppm (circa 520 mg/kg bw/day) produced marked toxicity in the dams, particularly during lactation where maternal body weight loss was observed. During mating, food consumption was decreased (9-15% in males and 5-11% in females) in both sexes at 5000 ppm with corresponding reduction in body weight gain (13% in males and 11% in females. During gestation body weight gain was 10-17% lower at 5000 ppm and during lactation food consumption was 21-23% lower than controls and dams lost body weight with a 0.2-3.3 g loss in body weight compared to gains of 7.9-21 g.

Although tremor and hypersensitivity and reduced forelimb grip strength were observed in most high dose dams of both generations (no clinical signs of toxicity were observed in males), these findings only occurred during lactation when there was clear evidence of marked systemic toxicity including maternal weight loss. 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. It should also be noted that before reaching the lactation phase on this study, animals would have been dosed for at least 90 days or more hence any observations during lactation cannot be considered to be a consequence of a single exposure. Refer to Table 15 in the attachment for further details.

The findings of the 2-Generation reproductive toxicity study in rats reflect marked

maternal toxicity after repeated exposure and do not provide any evidence of specific target organ toxicity that would meet the criteria for classification with STOT-SE2

In the developmental neurotoxicity study in rats (BASF Doc ID 2006/1031894; in-life phase from March 2005 to May 2005), doses of 15, 30 and 60 mg/kg bw/day were administered to pregnant females from gestation day 6 to post-natal day 21 with pups dosed directly from day 11 to day 21 post-partum. Upon commencement of direct dosing of pups there was significant pup mortality at the top dose with 22 pup deaths seen from days 5-14 post-partum. The auditory startle maximum amplitude and startle time to peak amplitude were as decreased in male pups in all dose groups compared to control which was due to a high average maximum amplitude value in controls exceeding the historical control range (studies dated from 1997-2003) as one control male had an exceptionally high value. Values for auditory startle maximum amplitude (sub-set 2) on post-partum day 24 were 549, 356, 391 and 354 for the control and respective dose groups. This compared to the historical range of 352-434 for day 24 post-partum. However, on post-partum day 60 values were 1669, 1620, 2104 and 1549 (historical range 629-1758) indicating the variable nature of this end-point. Similarly, in females on post-partum days 24 (411, 454, 384 and 425; historical range 294-507) and 60 (919, 1062, 1167 and 955; historical range 243-1688) values were generally higher than control, again reflecting variability in these measurements. Values (msec) for time to peak amplitude in males were 34.9, 28.9, 30.8 and 28.1 in controls and respective dose groups day 24 post-partum (historical range 24.6-35.4) and 42.6, 36.6. 37.7 and 36.3 on day 60 post-partum (historical range 30.2-42.5). In females time to peak amplitude values for post-partum day 24 were 32.5, 30.3, 29.8 and 26.9 for controls and the respective dose groups (historical range 27.5-36.1) and for post-partum day 60 were 31.9, 30.4, 34.4 and 29.8 (historical range 29.1-38.5). None of these observations are treatment related. Refer to Table 16 in the attachment for further details.

In a follow-up study (BASF DocID 2006/1031827) to investigate pup mortality during direct dosing on Days 11-21 post-partum, to replicate the conditions of the DNT study, pups were dosed at 0, 30, 60, 120 and 2000 mg/kg bw/d. Acute mortality and marked clinical signs were observed in pups at 120 and 200 mg/kg bw/d. Mortality at 60 mg/kg bw/d was comparable to that seen in controls. There were no effects at 30 mg/kg bw/d. Refer to Table 17 in the attachment for further details.

The DNT study provides no evidence of any neurological findings or any other finding that would support classification with STOT-SE2.

The DS cite the observation of sedation at the top dose of 3000 ppm in the non-GLP 3-month dog study conducted in 1977 (BASF DocID 77/035) as part of the justification for STOT-SE2. This was seen initially in all dogs for up to 4 weeks and then in 3/8 dogs on occasional study days thereafter. However, sedation was not observed in either of the two more recent 1-year dog studies (top doses of 1800 or 8000/6000 ppm; BASF Doc IDs 89/0357 & 94/10282) nor in the 28 day dog study (top dose 12,000 ppm; BASF DocID 94/10283), indicating that this was not a reproducible finding. The observation of sedation in a study judged "Supportive" does not provide a weight of evidence to support classification with STOT-SE2.

The DS also cite the observation of salivation in the 28 day (BASF Doc ID 94/10283) and

1 year dog studies (BASF Doc ID 94/10282) in support of the STOT-SE proposal (p. 85-86). Salivation observed at 2-6 hours post dosing in 28 day (BASF Doc ID 94/10283) and 1 year (BASF Doc ID 94/10282) dog studies at doses of 6000 ppm (166 mg/kg bw/d) and above. No incidence of salivation was recorded in the 3-month dog study (BASF Doc ID 77/035) up to a dose of 3000 ppm nor in the other 1 year dog study (BASF Doc ID 89/0357) up to a dose of 1800 ppm. The observation of salivation in the 1 year dog study occurred at a dose that caused deaths in 3 dogs (1/6 males and 2/6 females) which required the top dose to be reduced from 8000 ppm to 6000 ppm. Thereafter, marked clinical signs of toxicity were observed in 1 female at 6000 ppm from Day 7 onwards which required the animal to be sacrificed prematurely. Within the context of the mortality seen at 8000/6000 ppm in the 1-year dog study, the observation of post dose salivation, which in itself is a frequent observation in dog feeding studies, is not considered to indicate significant or severe toxicity after a single exposure and therefore does not meet the criteria for STOT-SE2. Overall Conclusion Regarding proposal for STOT-SE2 In conclusion, the criteria for classification with STOT-SE2 are not met as there is no convincing or reproducible weight of evidence to indicate that mepiquat-chloride can cause specific target organ toxicity (in this case neurotoxicity) after a single exposure at doses that are not associated with the classification for acute toxicity. The effects cited as supportive of this classification, many of which occur after repeated dosing, occur at doses that cause mortality. The effects seen in acute studies by the oral and inhalation routes are adequately described by the proposed classifications for acute toxicity i.e. Acute Tox. 3, H301 and Acute Tox. 4, H332. For further explanations including tabular overviews please refer to the attachment to the comments (BASF DocID 2020/2081825) ECHA note – An attachment was submitted with the comment above. Refer to public attachment Mepiquat Response to Public Consultation for CLP 28 May 2020_Attachment 2020_2081825.pdf
Dossier Submitter's Response
Thank you for your comprehensive comments. As stated in the CLH report we consider the neurotoxic effects observed for this substance borderline between no classification and classification for STOT SE. Some signs of neurotoxicity in response to single exposure were 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, also 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 LD50 and and LC50 values for the substance, i.e 50-300 mg/kg bw (Acute Tox 3) and 1-5 mg/L (Acute Tox 4). We agree that double classification for the same effects should be avoided and acknowledge that some signs observed at nonlethal doses may also be related to general toxicity or be due to experimental set up of the inhalation study. Therefore RAC should carefylly consider whether classification for STOT SE 2 (nervous system) is warranted.

RAC's response
Thank you for your comments. The toxicological profile of mepiquat chloride is characterised by acute lethality (especially in gavage studies), transient clinical signs indicative of neurotoxicity without histopathological alterations in the nervous system, and body weight depression at higher doses in dietary studies. An *in vitro* study demonstrating partial affinity of mepiquat to the nicotinic acetylcholine receptor (Franke, 1991) provides a possible mechanistic explanation of mepiquat-induced neurotoxicity. RAC is of the view that nervous system is a target organ of mepiquat and that the effects, albeit reversible, are in principle relevant for a STOT SE classification. At the same time, RAC acknowledges that the neurotoxic effects occurred mostly at or just below dose levels associated with mortality. RAC agrees that the effects at doses associated with mortality are covered by the acute toxicity classifications. 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.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	21
Comment received				
FR: FR agrees with the proposal of classification for environmental hazards (Aquatic chronic 3, H412)				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. Mepiquat Response to Public Consultation for CLP 28 May 2020_Attachment 2020_2081825.pdf [Please refer to comment No. 6, 11, 20]
2. MEPIQUAT CHLORIDE (ISO); 1,1-DIMETHYLPIPERIDINIUM CHLORIDE.pdf [Please refer to comment No. 3]