Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one

EC Number: 262-661-3
CAS Number: 61213-25-0

CLH-O-0000001412-86-242/F

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-242/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: **3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one**

EC Number: **262-661-3**

CAS Number: **61213-25-0**

The proposal was submitted by **Spain** and received by RAC on **29 September 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Spain 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 **5 December 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 February 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **Steve Dungey**

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 **30 November 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one	262-661-3	61213-25-0	Repr. 1B Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360Df H302 H317 H400 H410	GHS07 GHS08 GHS09 Dgr	H360Df H302 H317 H410		M=100 M=100	
RAC opinion	TBD	3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one	262-661-3	61213-25-0	Repr. 1B Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360DF H302 H317 H400 H410	GHS07 GHS08 GHS09	H360DF H302 H317 H410		oral: ATE = 500 mg/kg bw M=100 M=100	
Resulting Annex VI entry if agreed by COM	TBD	3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one	262-661-3	61213-25-0	Repr. 1B Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360DF H302 H317 H400 H410	GHS07 GHS08 GHS09	H360DF H302 H317 H410		oral: ATE = 500 mg/kg bw M=100 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

3-Chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one (flurochloridone) is a herbicide approved as an active substance in plant protection products (Directive 91/414/EEC). Currently, there is no entry in Annex VI of CLP regulation for flurochloridone. Therefore, the proposal of the dossier submitter (DS) addressed all physical, human health and environmental hazard classes.

The substance is a mixture, with a ratio of (1RS,2RS)(trans)- and (1RS,2SR)(cis)-isomers of approximately 3:1. All studies were performed using this mixture unless stated otherwise.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS did not propose classification for physical hazards. The data on physico-chemical properties did not indicate any concerns and as such, flurochoridone does not meet the criteria for classification.

Comments received during public consultation

There were no comments regarding the classification for physical hazard classes.

Assessment and comparison with the classification criteria

A test performed according to methods EEC A.10 showed that flurochloridone is not highly flammable. In addition, the structural formula of flurochloridone does not contain any of the chemical groups characteristic of explosive agents. Flurochloridone was not an oxidising solid according to the results of a report statement (Weissenfeld, 2006). Therefore, RAC is in agreement with the DS that **no classification is required for physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - Oral route

The CLH dossier included three acute oral toxicity studies. Two studies were performed in rats and one in mice. All studies were considered acceptable by the DS. In the first rat study, similar to OECD TG 401 (non GLP), the LD_{50} was 4000 mg/kg bw in males and 3650 mg/kg bw in females (Howell, 1979). In the second rat study, performed in females according to OECD TG 423 (GLP compliant), LD_{50} was found to be between 300 and 2000 mg/kg bw (Sieber, 2011). According to the DS, this lower LD_{50} range value might be attributed to the need to sacrifice the animals for ethical reasons in the latest study. Changes in ethical treatment of test animals that occurred between 1980's studies and more recent studies might justify the different results. After oral administration of flurochloridone in mice the LD_{50} was higher than 5000 mg/kg bw in both sexes (Ullmann, 1985).
The DS proposed classification for acute oral toxicity as Acute Tox. 4; H302 on the basis of the lowest oral LD_{50} obtained (Sieber, 2011) with an ATE of 500 mg/kg bw.

Acute toxicity - Dermal route

In an acute dermal toxicity study, similar to OECD TG 402 (non GLP) with some deviations, the LD_{50} was higher than 5000 mg/kg bw in rabbits in both sexes (Howell, 1979). The deviations were not considered to impact the results of the study. Thus, no classification was proposed by the DS.

Acute toxicity - Inhalation route

The results of a single acute inhalation toxicity study (OECD TG 403, GLP compliant) were presented by the DS (Decker, 2004). Rats (5/sex/dose) were exposed nose-only to 4.821 mg/L of flurochloridone for 4 hours. No deaths and no clinical signs were observed. It is not stated in the original study report whether this concentration was the maximal attainable concentration. Nevertheless, the DS argued that no mortality is expected to occur between 4.821 mg/L and the cut-off value for classification of 5 mg/L. Therefore, based on the available data, no classification was proposed.

Comments received during public consultation

One MSCA supported the proposed classification.

Assessment and comparison with the classification criteria

Acute toxicity - Oral route

RAC agrees with the DS that flurochloridone fulfilled the criteria for **Acute Tox. 4; H302** based on the range value of LD_{50} obtained in the Sieber (2011) study ($300 < LD_{50} < 2000$ mg/kg bw). For the converted acute toxicity point estimate, the conversion value obtained from table 3.1.2 of CLP regulation that relates to the results of the value of the available range test is **ATE = 500 mg/kg bw**.

Acute toxicity - Dermal route

Taking into account that the dermal LD_{50} in rabbits was above the threshold value of 2000 mg/kg bw triggering classification, RAC agrees with the DS, that **no classification for acute dermal toxicity** is warranted.

Acute toxicity - Inhalation route

No mortality was observed after exposure to the tested concentration of 4.89 mg/L flurochloridone (aerosol), which was the only dose tested. Although 4.89 mg/L is slightly below the cut-off value of 5 mg/L triggering classification, RAC agrees with the DS that as neither mortality nor clinical signs were observed in the study, **no classification for acute inhalation toxicity** is warranted**.**

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

Summary of the Dossier Submitter's proposal

In the available acute toxicity studies, no specific organ effects were observed after single acute exposure via the oral, inhalation or dermal route. On this basis, no classification was proposed by the DS.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

No evidence of specific toxic effects at any target organs or tissues and no signs of respiratory tract irritation or narcotic effects were observed in the available studies performed with flurochloridone. Accordingly, RAC agrees with the DS that **no classification for STOT SE** is warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of flurochloridone was investigated in one study in rabbits. The study was considered acceptable although it was performed according to US EPA TG of 1978, and deviated from OECD TG 404 (Howell, 1979). The study was not GLP. The test substance was applied undiluted for 24h to both abraded and intact skin in six rabbits. Skin reactions were recorded immediately after exposure and at 48h after patch removal. Only slight transient irritation (grade 1) was observed directly after exposure in one out of six animals. At 48h time point no reactions were observed in the six rabbits.

Despite the deviations from OECD TG 404 (24 instead of 4 hours exposure, abraded skin), the study was accepted by the DS. Based on the available data no classification was proposed.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

Flurochloridone was tested in a rabbit skin irritation study (Howell, 1979). The study was similar to OECD TG 404 with some limitations:

- 24h exposure instead of 4h exposure;
- observation 48h after patch removal instead of 24, 48 and 72h;
- test material was applied on abraded and intact skin;
- only 3 days of observation period;
- no vehicle was used and the test material (solid) may not have been moistened before application.

RAC notes that there is some uncertainty concerning the use of the study for classification since the laboratory did not use a vehicle and may not have moistened the solid test material. Nevertheless, some of these deviations (24 h exposure, abraded skin) might be considered as a worst case and might have increased the exposure to the test material. The only slight reversible irritation observed in one out of six animals supports no classification for flurochloridone. Moreover, the lack of any irritation in the eye irritation study is also in support of no classification for skin irritation. Therefore, RAC agrees with the DS that **no classification for skin corrosion/irritation** is warranted for flurochloridone.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In the single available eye irritation study performed according to US EPA (1978) guideline (non GLP), no eye irritation effects were observed after ocular application of flurochloridone to the eyes of nine rabbits (Howell, 1979).

Therefore, the DS proposed no classification for eye irritation.

Comments received during public consultation

No specific comments were received during public consultation.

Assessment and comparison with the classification criteria

The eye irritation study in rabbits was similar to OECD TG 405 with some limitations: no records of scores 1 hour after exposure and 3 animals were rinsed after instillation. The cornea, iris and conjunctiva were not affected by instillation of the undiluted test material (all scores were zero at all time points) in the nine rabbits (with unwashed or rinsed eyes).

Considering that no eye irritation was observed, RAC agrees with DS that **no classification for serious eye damage/irritation** is warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Two GLP studies were available for this endpoint. The first is an open epicutaneous test carried in guinea-pigs (Mutter, 1985). The study was negative but was not considered acceptable by the DS because the highest dose of 30%, chosen for the induction and challenge phases, did not induce mild irritation. The second study, a Guinea Pig Maximisation Test (GPMT), was performed according to OECD TG 406 (Arcellin, 2006). In this study, flurochloridone was found to be a skin sensitiser since a skin reaction was observed in 100% of the animals (10/10) in the test group after challenge (0% in control group) at an intradermal induction concentration of 25% and at a challenge concentration of 5%. Therefore, the DS proposed to classify flurochloridone as Skin Sens. 1; H317. Because data with lower intradermal induction concentration than 25% were not available, it was not possible to exclude category 1A and therefore no sub-categorisation was proposed by the DS.

Comments received during public consultation

No specific comments received during public consultation.

Assessment and comparison with the classification criteria

No human data on skin sensitisation of flurochloridone are available. RAC agrees that the open epicutaneous test is not suitable as dose levels were insufficient for both induction and challenge. In an acceptable GPMT study, positive results were observed in 100% of the tested animals. Therefore, flurochloridone fulfilled the criteria for classification as a skin sensitiser. With regard to sub-categorisation, flurochloridone fall within category 1B: ≥ 30% of the animals responding at > 1% intradermal induction dose. Nevertheless, there is no study investigating the sensitising properties of flurochloridone at intradermal induction concentrations needed to exclude category 1A (i.e. ≤ 1%). Thus, in the absence of such data the CLP regulation specifies that the skin sensitising substance shall be classified in category 1 without a subcategory. Therefore, RAC agrees with the DS to classify flurochloridone as **Skin Sens. 1; H317** without sub-categorisation.

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

Summary of the Dossier Submitter's proposal

The evaluation of the specific target organ toxicity – repeated exposure (STOT RE) hazard point was based on two dietary repeated dose toxicity studies in rats (Ouelette, 1982 and Ouelette, 1982b), one in mice (Ouelette, 1982a) and one in dogs (Blair, 1983). All studies followed OECD TG protocols and were conducted according to GLP. In addition, the dietary fertility toxicity study conducted in male rabbits (Wilcynski and Killinger, 1985c; non-guideline) was also considered relevant for evaluation of STOT RE.

According to the DS effects considered as impairments in organs were generally seen at the top dose. They corresponded to adaptive findings and were not of toxicological significance at a dose below cut-off triggering STOT RE classification. The only adverse effect that was observed at a dose level relevant for classification as STOT RE 2, was the non-reversible extramedullary

haematopoiesis observed in liver in the male rabbit fertility toxicity study. The study report suggested that this effect may have been a sign of early cirrhotic changes.

***Table 1**: Liver findings observed in the rabbit fertility study (Wilcynski and Killinger, 1985c)*

	Liver, incidence (grading) n=6 rabbits *(12.9 ≤ STOT RE 2 ≤ 129 mg/kg bw/day)*			
Dose (mg/kg bw/day)	0	1	5.9	33.9
After 10 weeks treatment				
Biliary hyperplasia	0	0	0	4 (1.3)
Extra medullary haematopoiesis	1 (1.0)	3 (1.1)	1 (1.0)	4 (1.3)
After 5 weeks recovery				
Biliary hyperplasia	2 (1.0)	1 (1.0)	1 (1.0)	2 (1.5)
Extra medullary haematopoiesis	3 (1.0)	1(1.0)	3 (1.0)	5 (1.2)

Grade: 1=minimal, 2= mild

Nevertheless, the DS noted that the dose-dependency was not clear, the severity of the lesion was graded from minimal to mild and these liver findings were not found in other species such as mice, rats and dogs. Thus, the DS concluded that these liver effects are insufficient evidence to support a classification of flurochloridone as STOT RE.

Other studies on carcinogenicity and reproductive toxicity were not further considered for STOT RE because the observed effects were either covered by other endpoints (effects on reproductive organs) or not considered of toxicological significance below the cut-off values.

Overall, the DS concluded that classification for STOT RE is not warranted.

Comments received during public consultation

No specific comments received during public consultation.

Assessment and comparison with the classification criteria

No human data are available with flurochloridone. Animal data are only available by oral route.

Rats

In the 21d range-finding toxicity study in rats (Ouelette, 1982), the main effects were observed in the liver (increased relative liver weight, bilirubin, cholesterol, gamma-GT and alkaline phosphatase), in the kidney (increased relative weight and blood urea nitrogen concentration) and in the haematopoietic system (spleen discoloration, decreases in leukocyte and erythrocyte counts and in haemoglobin and haematocrit). Effects on the reproductive tract of males and females were only observed at top dose (1017 and 1226 mg/kg bw/day respectively). At a dose that would be relevant for classification (230.6 and 242.4 mg/kg bw/day respectively), a statistically significant increase in liver weight (< 10%) and in cholesterol was observed in both males and females. At this dose, discoloration of liver and spleen were only seen in few animals. RAC considers that these findings do not fulfil the classification criteria.

In the 90-day rat toxicity study, the main findings were the increase in liver weight without histopathological findings and reproductive organs changes at top dose (137.5 mg/kg bw/day) in males and at ≥ 31.4 mg/kg bw/day in females. The effects observed in the ovaries at 31.4

mg/kg bw/day are relevant for classification and will be discussed in the reproductive toxicity section.

In the combined rat chronic toxicity/carcinogenicity studies, in the rat mechanistic studies and in the 2-generation rat toxicity study, no relevant findings were observed above cut-off triggering STOT RE classification.

Overall, based on the rat repeated-dose toxicity studies, classification is not required.

Mice

Effects observed in the 28-day range-finding study (Ouelette, 1982a) and in the carcinogenicity study (Sprague, 1985b) were above the upper limit for STOT RE 2 classification (300 mg/kg bw/day for a 4-week study, 12.5 mg/kg bw/day for a 2-year study). Thus, based on the mice repeated dose toxicity studies, classification is not warranted.

Dogs

In the available 6-month dog repeated dose toxicity study, at the top dose level of 1000 ppm (30 mg/kg bw/day for male), relevant for classification (5 ≤ STOT RE 2 ≤ 50 mg/kg bw/day), the decreases in haematocrit and haemoglobin observed in the study were not associated with other changes and therefore are considered insufficient to fulfil the criteria. In the same study, occasionally irregular heart rate was observed in some animals (incidence not specified) in both sexes. As the effect decreased towards the end of the treatment and was not associated with histopathological changes, irregular heart rate is not considered for fulfilling the criteria for classification as STOT RE 2.

Rabbits

In the fertility study (Wilcynski and Killinger, 1985c), males rabbits received flurochloridone at 1, 5.9 and 33.9 mg/kg bw/day in their diet. Liver weight and clinical chemistry were not investigated in this study. An increase incidence of minimal to mild hepatic biliary hyperplasia was seen in liver of 4/6 males at top dose (within the guidance values for classification as STOT RE 2). The lesion was sometime associated with periportal fibrous connective tissue and mononuclear cell infiltrates. After 5-week recovery, 2 males still showed slight to mild biliary hyperplasia. Slight biliary hyperplasia were also observed in 2 control males after recovery. Overall, the toxicological relevance of the finding is doubtful and this effect is not considered of sufficient concern for classification. With regards to the increase in extra medullary haematopoiesis observed at top dose in this study, RAC agrees with the DS that as no dose-response was observed and as no changes in haematological parameters was noted, extramedullary haematopoiesis is not of sufficient concern for classification. In conclusion, RAC agrees with the DS's proposal that flurochloridone **does not warrant classification as STOT RE**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The CLH dossier of flurochloridone included a battery of *in vitro* tests performed according to OECD TGs and were GLP compliant. Flurochloridone was negative in Ames tests. A negative result was also obtained in an *in vivo* gene mutation assay in L5178Y mouse lymphoma cells. Equivocal results were obtained in two *in vitro* tests: cytogenetic and micronucleus tests. With regard to DNA damage and repair (non-guideline and GLP), negative results were obtained *in vitro* in an unscheduled DNA synthesis tests and DNA repair activity test.

In vivo, a negative micronucleus assay (OECD TG 474) with flurochloridone was available.

Other non GLP pre-guideline studies were reported by the DS but were only considered as supplementary information. In addition to being different from the current guideline, the purity of flurochloridone was lower in those studies and the composition less representative of current technical flurochloridone used in the more recent guideline studies. Overall, the DS concluded that no classification for germ cell mutagenicity is warranted for flurochloridone.

Comments received during public consultation

One MSCA questioned the proof of bone marrow exposure in the negative guideline *in vivo* micronucleus assay. The DS responded that in the toxicokinetic studies in rats, residual radioactivity was observed in bones after single doses of 4 or 200 mg/kg bw flurochloridone. The DS added that decreased white blood cells in the carcinogenicity study could have been secondary to bone marrow exposure and alteration.

Assessment and comparison with the classification criteria

In vitro

Four Ames assays were performed with flurochloridone at up to 5000 µg/plate. Two tests were performed according to OECD TG 471 (GLP compliant) and two tests were performed following a protocol prior to the guideline. The four assays were negatives with and without metabolic activation (Sokolowski, 2008 and 2011; Jagannath, 1978a, b).

Two gene mutation assays were available on mouse lymphoma cells. The most recent test was performed according to OECD TG 476 (GLP compliant) and was negative (Wollny, 2008). The second test (Matheson, 1978) was performed following a protocol prior to the guideline. It was non GLP and it used a batch of flurochloridone of lower purity. In this study, an increase in the number of mutants was observed in presence of metabolic activation at 60 and 90 µg/mL. At these doses, cytotoxicity was not too high as relative cloning efficiency was 69% and 96% of control at 60 and 90 µg/mL, respectively. Nevertheless, these results are difficult to interpret as there were no statistical analysis performed, no historical control data and no repeated experiment. As the slight increase in mutations (less than factor 2 compare to control) occurred without dose-response relationship, the results are considered equivocal and of lower weight than the negative results observed in Wollny, 2008.

Two guideline studies investigated chromosomal mutations. In the *in vitro* chromosome aberration assay (Hoffmann, 2008) performed in V79 cells of Chinese hamster, a statistically significant dose-related increase in polyploid cells was observed without metabolic activation. However, this effect was not reproduced in a second experiment. A statistically significant increase in aberrant cells was observed with and without metabolic activation in the first and the second experiment at least at one dose tested. Except in the second experiment (with metabolic activation) the number of aberrant metaphase was within historical control range. As no dose-response was observed, the positive results of the study are considered equivocal. RAC notes that in this study dose levels may have been too low as cytotoxicity was not high enough (mitotic index well above 50% of control in most of the experiments). In the *in vitro* micronucleus test (Bohnenberger, 2012), a statistically significant increase in micronucleus was observed at 100 and 200 µg/mL without metabolic activation (after 4h exposure) but was not dose-related and in presence of precipitation of test material. Nevertheless, the increase was reproduced in a second experiment (24h exposure) at 25 µg/ml without precipitation. Therefore, the test is considered positive in absence of metabolic activation. Historical control data were not reported in the CLH dossier.

No indication of effects on DNA repair in human fibroblasts or in an unscheduled DNA synthesis in Hela cells was observed (Pirovano, 1986; Snyder, 1985). Nevertheless, the relevance and weight of these studies are low (the OECD TG of the *in vitro* UDS has been withdrawn since 2014). Flurochloridone was also negative in an *in vitro* cell transformation assay in mice cells.

In vivo results

There were two *in vivo* micronucleus tests available with flurochloridone, one in rat and one in mice. In rats, the study was performed according to OECD TG 474 and was negative up to the limit dose of 2000 mg/kg bw (Roth, 2012). In this study, no proof of exposure to the bone marrow is available. Nevertheless, according to the available toxicokinetic data, flurochloridone is expected to reach the bone marrow.

In the assay performed in mice (non-guideline and non GLP), three dose levels were used up to 5000 mg/kg bw (Majeska, 1985). RAC noted that in this study, the two highest dose levels were above the recommended limit dose of 2000 mg/kg. Moreover, this micronucleus assay was performed with a test batch of lower purity than the batch used in the *in vivo* micronucleus assay in rats (Roth, 2012). A statistically significant increase in micronucleus was observed at 1250 mg/kg bw and 2500 mg/kg bw in males. However, the observation was not reproduced in a second experiment. No effects were observed in females but the positive control was only positive in male and not in female rats. Overall, RAC considers the result of this study equivocal.

Comparison with the classification criteria

Overall, although positive results were observed *in vitro*, a negative in *vivo* mammalian micronucleus test is available. Although no proof of bone marrow exposure was available in the study, toxicokinetic study available with flurochloridone suggests than bone marrow can be exposed after exposure to flurochloridone. The equivocal results obtained in the micronucleus test performed in mice (Majeska, 1985) are considered of lower weight than the negative results obtained in the more recent study of Roth (2012). Therefore, RAC agrees with the DS that **flurochloridone does not meet the criteria for classification as a germ cell mutagen**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Carcinogenicity potential was tested in rats and mice following administration of flurochloridone for two years.

In the rat study (Sprague, 1985a, OECD TG 453, GLP compliant), no neoplastic lesions were observed.

In the mouse study (Sprague, 1985b, OECD TG 451, GLP compliant), mice were tested during two years at 0, 6.3/9.1, 25.7/32.1, 100.1/143.5 mg/kg bw/day in males/females, respectively. No mortality or clinical signs were associated with treatment. Decrease body weight gain (not statistically significant) was observed at the highest dose only. With regards to neoplastic findings, hepatocellular carcinomas were increased in males at top dose and adrenocortical adenomas were increased in males at all doses tested. With regards to non-neoplastic findings, a marked increase in relative weight of adrenals was observed in both male and females at ≥ 25.7 mg/kg bw/day. At interim sacrifice but not at terminal sacrifice, liver weight was increased at ≥ 25.7 mg/kg bw/day. Moreover, at the highest dose, liver necrosis was increased in males. According to the DS in most cases the liver foci of necrosis were secondary to neoplastic or inflammatory processes.

The increase in hepatocellular carcinoma was not considered related to treatment by the DS as dose-response was unclear, tumour incidence was comparable to that observed in other laboratories at similar time and as the increase was not statistically significant.

The increase in adrenocortical adenomas were also not considered related to treatment as no dose-response relationship was observed. Moreover, there was a higher incidence of adrenocortical adenomas above historical control values even in control animals. This may have been explained by the higher duration of the study (24-month instead of 18-month recommended for mice).

***Table 2**: Incidence of liver necrosis and selected neoplastic findings*

	Pathological findings (%)			
dose (ppm)	0	50	200	800
Liver necrosis				
Males	1.7	3.3	5.0	12
Females	13	18	10	8
Hepatocellular adenoma				
Males	41	38	45	40
Females	15	15.3	16.6	18.3
Hepatocellular carcinoma				
Males	16.6	11.6	18.3	26.6
Females	5	3.3	0	6.9
Adrenal cortical adenoma total				
Males	45.0	58.3	44.8	51.7
Females	5	3	1.6	0

***Table 3**: Comparison of Sprague (1985b) study results with available historical controls data in B6C3F1 mice for hepatocellular carcinoma and adrenocortical adenoma*

	Hepatocellular carcinoma	**Adrenocortical adenoma**
Sprague (1985b) (males)	16/60 (26.6%) at 800 ppm	27/60 (45%) at 0 ppm 35/60 (58.3%) at 50 ppm 26/58 (44.8%) at 200 ppm 31/60 (51.7%) at 800 ppm
Charles River Laboratories (1979-86) Animal breeder, sex not specified	4.2-24.6%	-
Huntington Life Sciences (1985-2000) Animal supplier, sex not specified	4.0-38.8%	-
Haseman *et al.* (1984) (males) NTP database on 51 studies until march 1983	498/2334 (21.3%)	53/2240 (2.4%)
Study conducted at similar time (DAR) (males)	13/60 (21.6%)	3/60 (5%)

Based on the results of the studies in rats and mice performed with flurochloridone, the DS concluded that in rats there was no evidence of carcinogenicity at any tested dose levels, while in mice, the overall weight of evidence is considered insufficient to justify a classification for carcinogenicity. Thus, no classification was proposed by the DS.

Comments received during public consultation

One MSCA supported no classification for carcinogenicity but requested clarification on the statistically significance of liver cell carcinoma and historical control ranges of adrenocortical

adenoma. The DS responded that the increase in liver cell carcinoma was not statistically significant and that the historical control range for adrenocortical adenoma was not available.

Assessment and comparison with the classification criteria

RAC agrees that no evidence of tumours were observed in the rat 2-year study. Nevertheless, RAC noted that relatively low dose levels were tested in the study (only up to 15.7 mg/kg bw/day in males and 19.3 mg/kg bw/day in females). Indeed, at the top dose, no clinical signs were observed and body weight decrease was less than 10%. Moreover, although body weight gain decreased (10-15%), the effect was not statistically significant.

In the 2-year mice study, several limitations were noted:

- biochemical parameters were not investigated;
- study duration was 24-month instead of 18-month. Nevertheless, the higher duration of the study had no impact on the survival rate as it was higher than 50% in all tested group except at 25.7 mg/kg bw/day in males.
- the highest dose level may have been too low, as no evidence of toxicity was observed at this level. There was no statistically significant decrease in body weight gain or in body weight in the study. No effects on food consumption were reported in the dossier.

The most relevant finding was an increase number of tumours in liver and adrenal glands.

Liver tumours

An increase in hepatocellular carcinoma was observed in male mice (see table 2 above). The increase was not statistically significant using Matel-Haenszel 2x4 trend analysis. Nevertheless, no analysis has been performed using pairwise comparisons with concurrent controls.

With regards to non-neoplastic findings in liver, a dose-related non-statistically significant increase in liver necrosis was observed in males (see table 2).

The incidence in hepatocellular carcinoma in males (26.6%) at the high dose was slightly above the historical control range obtained from Charles River laboratories (studies initiated between 1979-1986) with a mean incidence of 13.2% and a range of 4.2-24.6% (sex not specified). The increase was also above the incidence of 21.6% observed in a study conducted at a similar time period (time not specified in the CLH dossier). The incidence was reported to be inside the historical control data from the animal supplier in the same strain of mice in the time 1985-2000 (Huntington Life Sciences). The studies showed a maximum increase in hepatocellular carcinoma of 4-39.8% with a mean of 20.03% and for hepatocellular adenoma a range of 1.9-32% (sex not specified). However, the relevance of this historical control data was questionable as the time period was very long (25 years) and it is not possible to know if an increase in this type of tumour has been observed over time. Finally, the incidence was also above the reported incidence from 51 NTP studies, but the historical controls may not be appropriate as the exact period of time was not specified.

No additional factors raised the level of concern. Female mice had low incidences of these types of tumours with no apparent dose-response relationships. There is no toxicokinetic data or mechanistic studies in support of significant differences in ADME between male and female mice that would explain differences in the carcinogenic potential of flurochloridone. No effects were observed in the carcinogenicity study in rats. The substance is not considered mutagenic. No further mechanistic investigation is available.

Overall, the increase in liver adenocarcinoma was only seen in one sex and at top dose hence it is considered insufficient to support a classification of flurochloridone.

Adrenal gland tumours

The increase in adrenal gland tumours was not statistically significant using Matel-Haenszel 2x4 trend analysis. Nevertheless, no analysis was performed using pairwise comparisons with concurrent controls.

With regards to non-neoplastic findings, a statistically significant increase in absolute and relative adrenals weight was observed in males at interim sacrifice but the values were comparable to controls at the end of the treatment.

The increase in adrenal cortical adenoma in male was above available historical control data (see table 3). Nevertheless, these historical control data are of low relevance as the concurrent control of the study was well above these historical control data (45% vs 2.4-5%). In the absence of dose-response and statistical significance, the finding is not considered sufficient to support a classification.

In conclusion, RAC supports the DS's view that **classification for carcinogenicity is not warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on fertility and sexual function

The evaluation on fertility and sexual function of flurochloridone was based on a two-generation study in rats (Downs and Minor, 1983, OECD TG 416, GLP compliant), a fertility study in male rats (Wilcynski, 1984, non-guideline, GLP), two mechanistic studies in male rats (Wilcynski, 1985a, b, non-guideline, GLP), a fertility study in male rabbits, (Wilcynski, 1985a, b, non-guideline, GLP) and an analysis of sperm production in nonhuman primates (Wilcynski, 1985d, non-guideline, GLP). In addition, repeated dose toxicity studies performed in rats were used by the DS (see summary of relevant effects in table 4 below).

In the 2-generation study in rats (Downs and Minor, 1983), there were clear effects on the reproductive performance at the top dose (1000 ppm equivalent to 70 mg/kg bw/day) in all parental animals. Effects were observed on mating index, gestation index and fertility index of all parental generations at this dose. The number of females with implant sites was also significantly reduced in P1 and P2. The pups born/litter, the live birth index and the live pups per litter on day 0 were significantly decreased in P1 to give F2 pups. Parental toxicity consisted of decreased body weight gain at this dose level. Male reproductive organs (testes and epididymides) were severely affected after treatment in all generations. The sperm analysis performed in P0 and P1 revealed significant increases of abnormal sperm with respect to controls from 400 ppm and significant reduction in the sperm motility at 1000 ppm in both generations. The cross mating of the 1000 ppm males with 0 ppm females showed an extreme reduction in the fertility index not observed in the cross mating of the 1000 ppm females with the 0 ppm males. This fact pointed to male specific fertility impairment.

Another fertility study in rats confirmed the male fertility effects observed with flurochloridone (Wilcynski, 1984). Two mechanistic studies were performed with flurochloridone (Wilcynski and Killinger, 1985a,b). In the first study, no effects were observed. This may be due to the short dosing period (only five consecutive days). In the second mechanistic study clear disturbance of the spermatogenic cycle was observed: increase in step 17, 18 and 19 spermatids, abnormal sperm, decrease sperm. Sertoli cells damage was also noted in the study. Increases in LH and FSH from week 6 were regarded as secondary to testicular toxicity by the DS.

In the repeated dose toxicity and carcinogenicity studies in rats, changes on male reproductive organs were also noted. The effects in testis included a decrease in testis weight (absolute, relative) and size, atrophy of seminiferous tubules and Leydig cells hyperplasia. The other main target organ was the epididymides (reduced size, degeneration of spermatic elements, microtubular hyperplasia).

Thus, the DS concluded that flurochloridone is a clear fertility toxicant in male rats. The effects observed in testes and epididymides, sperm and fertility parameters were considered by the DS as a direct consequence of the disturbance of the spermatogenic cycle as shown by a mechanistic study in rats. These findings were not secondary to parental toxicity. Similar effects on testis and epididymides were not observed in mice, dogs, and rabbits. Reproductive effects were observed neither in the fertility study in male rabbits nor on sperm analysis in non-human primate. The DS concluded that the evidence in male reproductive toxicity in male rats only suggest a specific sensitivity of this species. Nevertheless, as no mechanism of toxicity is available, the relevance to human cannot be excluded. Based on a weight of evidence the DS proposed to classify flurochloridone as Repr. Cat. 2; H361f "suspected of damaging fertility".

***Table 4**: Overview of relevant effects on sexual function/fertility parameters and reproductive organs*

Study design	Result	Reference
Multigeneration reproductive toxicity study in rats 0, 40, 400, 1000 ppm (eq. to 2.8/3.2, 27.7/31.6, 70/78.1 mg/kg bw/day in males/females, based on pre-mating)	↓* bw gain in males and females ≥ 1000 ppm **Testes** (≥ 1000 ppm) ↓* absolute weight (P0, P1, P2); ↑*incidence of small size and atrophy (P0, P1, P2); ↑*interstitial cell hyperplasia and vascular degeneration (P1, P2), dose-related from 400 ppm **Epididymides** (≥ 1000 ppm) ↑*incidence of small size, sperm degeneration and tubular epithelial hyperplasia; **Female reproductive organs** No evaluation in the study of cycles and follicles in the ovary, no uterus weight record. **Sperm parameters** ↓*sperm count, ↑* abnormal sperm (≥ 400 ppm); **Reproductive performance** ↓* male and female mating (P0, P1) (≥ 1000 ppm) ↓* male and female fertility index (P0, P1, P2) (≥ 1000 ppm) ↓* gestation index (P0, P1, P2) (≥ 1000 ppm) ↓* implant sites (P1, P2, not reported for P0) (≥ 1000 ppm) ↓* litter size (F1) (≥ 1000 ppm) ↓* live birth and viability index in F1, not statistically significant in F2 Table: Cross mating fertility indices	Downs and Minor, 1983

<table>
<tr><th>Study design</th><th>Result</th><th>Reference</th></tr>
<tr><td></td><td>
<table>
<tr><td></td><td colspan="4">P0</td></tr>
<tr><td>Males (ppm)</td><td>0</td><td>40</td><td>400</td><td>1000</td></tr>
<tr><td>Females (ppm)</td><td>1000</td><td>40</td><td>400</td><td>0</td></tr>
<tr><td>Females</td><td></td><td></td><td></td><td></td></tr>
<tr><td>cohabited</td><td>20</td><td>19</td><td>17</td><td>20</td></tr>
<tr><td>Pregnant</td><td>16</td><td>16</td><td>17</td><td>2</td></tr>
<tr><td>fertility index [%]</td><td>80.0</td><td>79</td><td>100</td><td>10.0</td></tr>
<tr><td>Males</td><td></td><td></td><td></td><td></td></tr>
<tr><td>placed with females</td><td>20</td><td>19</td><td>17</td><td>20</td></tr>
<tr><td>with females pregnant</td><td>16</td><td>16</td><td>17</td><td>2*</td></tr>
<tr><td>fertility index [%]</td><td>80.0</td><td>84</td><td>100</td><td>10.0*</td></tr>
</table>
* p < 0.05 (Fisher's exact test),
</td><td></td></tr>
<tr><td>Fertility study in male rats

0, 100, 600, 1000 ppm (eq. to 5.7, 35.8, 60.7 mg/kg bw/day)

20 male/group 10-week premating, Female not treated After 13-week recovery period, 10 male/group were mated with 20 new females</td><td>↓* Dose related bw gain and food consumption

Sperm analysis: ↓*sperm count, ↓* sperm motility, ↑* % sperm abnormalities (≥ 600 ppm), improve after recovery but still statistically significant at 1000 ppm;

Hormone levels: no effects on testosterone, ↑*FSH, ↑ LH (≥ 1000 ppm); no effects after recovery;

Testis: ↓* weight, histopathology, not reversible after recovery;

Epididymis: ↓* weight, histopathology (≥ 600 ppm), effects observed (≥ 600 ppm after recovery but not statistically significant

Prostate: ↑* lymphocytic inflammation

Reproductive performance
↓* mating index after 10-weeks (≥ 1000 ppm), not statistically significant after 13-week recovery
↓* fertility index after 10-weeks (≥ 1000 ppm), not statistically significant after 13-week recovery
↓* implantation index (after 10-weeks and after recovery) (≥ 1000 ppm)
↓* litter size (after 10-weeks) (≥ 600 ppm), not statistically significant after recovery</td><td>Wilcynski, 1984</td></tr>
<tr><td>Mechanism of action study in male rats

0, 20, 100, 400 mg/kg bw/day

Male mated on day 0. Treatment on day 7 during 5 consecutive days. Afterwards 10 weekly matings</td><td>↓bw gain and food consumption (400 mg/kg bw/day)

No effects on male fertility index or caesarean section (corpora lutea, implantation, viable foetuses, early resorption, late resorption)</td><td>Wilcynski and Killinger, 1985a</td></tr>
<tr><td>Mechanism of action study in male rats

0, 1000 ppm (eq. to 56.1 mg/kg bw/day)

10-week</td><td>The study was performed to determine the stage of the spermatogenic cycle affected in male rats.

↓* bw and food consumption

Testis: ↓* absolute weight (not reversible after recovery)
↓* sperm count in testis since week 6, change in spermatid release (decrease in stage 18 and 19 spermatids) and germ cell displacement since week 2 onward, later germ cell</td><td>Wilcynski and Killinger, 1985b</td></tr>
</table>

Study design	Result	Reference
7 rats/group sacrificed after 24, 48, 96 h of treatment and 1, 2, 4, 6, 8, 10 weeks after. Remaining groups sacrificed after 10, 20, 30 weeks of treatment	degeneration occurred along Sertoli cells vacuolisation. Only half of the animals recovered after the recovery period **Epididymis:** ↓* absolute weight (not reversible after recovery) ↑* Spermatid step 17 in week 2 and ↓*sperm count since week 6 and before in caput epididymides (week 4), ↓*sperm motility, ↑* % sperm abnormalities since week 6. All reported microscopic findings returned to normal during the recovery period of 31 weeks except for abnormal sperm and sperm count Immature germ cells and cellular debris since week 1. ↑FSH, LH from week 6, no effect on testosterone	
Fertility study in male rabbits 0, 35, 220, 140 ppm (eq. to 0, 1, 5.9, 33.9 mg/kg bw/day) First mating then 10-week exposure and remated. After remating, 6 males/group were necropsied for male reproductive tract and selected organ weights.	No effects on bw gain, ↓* food consumption during weeks 3-7, liver histopathological findings No effects on fertility No effects on sperm analysis	Wilcynski and Killinger, 1985c
Effects on sperm production in male monkey 5-day/week during 12 weeks Blood chemistry, sperm analysis (motility, concentration, morphology) 0, 1, 8, 64 mg/kg bw/day	At 64 mg/kg bw/day ↓* bw gain, ↓fc ↑TG, ↓* Hct, ↓*Hb. Cholesterol remained normal **Sperm analysis**: no relevant changes in sperm concentration, sperm mobility and sperm count. ↑* abnormal sperm on week 4. Considered incidental as also observed at 1 mg/kg bw/day at week 7. LH, FSH and testosterone within normal values.	Wilcynski and Killinger, 1985d
21d range-finding study in rats 0, 41.2, 91.7, 230.6, 624, 1017 mg/kg bw/day for males and 0, 44, 106.6, 242.4, 648.1 and 1226 mg/kg bw/day for females	1017/1226 mg/kg bw/day in male/females: **Prostate**: small **Testes**: small **Female genital tract**: hypoplasia (no further details)	Ouellette, 1982
90-d study in rats 5.4, 26.6 and 137.5 mg/kg bw/day for males and 6.2, 31.4 and 154.6 mg/kg bw/day for females	↓ bw gain and food consumption **Testes**: ↓* absolute and relative weight, histopathological findings (137.5 mg/kg bw/day) **Epididymides**: histopathological findings (≥ 137.5 mg/kg bw/day) **Seminal vesicles**: macroscopic findings (≥ 137.5 mg/kg bw/day)	Ouellette, 1982b

Study design	Result	Reference
	Ovaries: dose-related ↑* absolute /relative weight (23%/37% at 154.6 mg/kg bw/day and 26%/32% at 31.4 mg/kg bw/day). Not considered relevant by the DS because not accompanied by histopathological findings.	
Carcinogenicity study in rats 0, 1.5, 3.9 and 15.7 mg/kg bw/day for males and 0, 2.0, 4.8 and 19.3 mg/kg bw/day for females.	**Testis** : macroscopic and microscopic findings (15.7 mg/kg bw/day) **Epididymides**: macroscopic and microscopic findings (≥ 15.7 mg/kg bw/day)	Sprague, 1985a
6-month study in dogs 0, 1.7, 7.1 and 30.0 mg/kg bw/day for males and 0, 1.6, 7.3 and 32 mg/kg bw/day for females.	↓ bw gain in females (30% on week 26, not statistically significant). No effects on bw reported in males. No effects on reproductive organs in females/males up to 32 mg/kg bw/day.	Blair, 1983
28-day range-finding study in mice 8.3, 33.6, 122.5, 528.8, 1586.0 mg/kg bw/day for males and 9.2, 38.2, 137.2, 535.1, 1841.3 mg/kg bw/day for females	No effects in males/females up to 1586/1841.3 mg/kg bw/day, respectively.	Ouelette, 1982a
Carcinogenicity study in mice 0, 6.3/9.1, 25.7/32.1, 100.1/143.5 mg/kg bw/day in males/females	↓ bw gain in male and females **Ovaries**: dose-related ↑* absolute/relative weight (≥ 32.1 mg/kg bw/day) at interim but not at terminal sacrifice. Not considered toxicologically relevant by the DS as not observed at the end of the study and without concomitant histopathological findings.	Sprague, 1985b

*statistically significant; fc: food consumption

Developmental toxicity

Three prenatal developmental toxicity studies were available to the DS and considered acceptable. Two studies were performed in rats and one study was available in rabbits.

In the first teratogenicity study in rats (Nemec, 1983a), toxicity to the offspring was observed at all doses: 25, 100 and 400 mg/kg bw/day. Maternal toxicity was observed at ≥ 100 mg/kg bw/day including corrected body weight and clinical signs at 400 mg/kg bw/day. Reduced foetal weight was observed at ≥ 100 mg/kg bw/day. Increased incidences of visceral malformations (diaphragmatic hernia, heart/great vessel anomalies) were observed at all dose tested. Although no historical control data were available for this type of malformations, the malformations were dose-related and a trend was identified at mid and high dose levels. Other visceral malformations were seen at 100 mg/kg bw/day and 400 mg/kg bw/day (malpositioned incisors, undescended testes, retinal folded, malpositioned ovaries, thymus absent). Visceral variations were significantly increased at ≥ 100 mg/kg bw/day. Skeletal malformations and variations were observed from 100 mg/kg bw/day. Exoccipital-cervical vertebrae defect was identified as the main significant skeletal malformations at ≥ 100 mg/kg bw/day by the DS. External malformations were observed at 400 mg/kg bw/day (Omphalocele).

In the second teratogenicity study in rats (Nemec, 1984a), lower dose levels were used: 0.2, 2, 10, 20, 100 mg/kg bw/day. Maternal toxicity (↓ 13% body weight gain on GD0-20) was observed at 100 mg/kg bw/day. At 100 mg/kg bw/day, a significant decrease in foetal body weight and a significant increase in both skeletal and visceral malformations were reported.

In the developmental toxicity study in rabbits, the doses tested were 0, 5, 20 and 60 mg/kg bw/day. Offspring toxicity occurred at the top dose of 60 mg/kg bw/day in presence of maternal toxicity (clinical signs, decreased bw gain and food consumption). An increase in post-implantation losses and 2 total resorptions were observed and considered secondary to maternal toxicity. The main clear effect was a significant increase in the incidence of sternebrae thread-like attached and hyoids arches bent (variation) at the high dose tested. These results were outside the historical control range for foetuses but were considered non-significant. At this dose, a higher incidence in major blood vessel variation was also observed (non-significant, within the range of historical controls for both foetuses and litters). The DS questioned the relevance of the maternal toxicity observed in the study as the corrected body weight reduction were exiguous and as absolute weights remained at the levels comparable to controls. Rat seems to be a more sensitive species and the tested dose levels in rabbit are not considered to be sufficiently high to rule out severe effects on development in this species and thus the teratogenicity potential of flurochloridone in rabbit cannot be discarded.

Overall, clear evidence of teratogenic effects were observed in rats. Malformations were not considered secondary of maternal toxicity. Therefore, the DS proposed to classify flurochloridone as Repr. 1B for developmental effects.

Comments received during public consultation

Fertility

One MSCA proposed Repr. Cat. 1 B for fertility based on the severity of the effects observed in the male rats at low dose levels, the unknown mechanism and the absence of data supporting non-human relevance.

One MSCA supported the DS' proposal for category 2 for fertility as category 1B was already proposed for developmental toxicity. Nevertheless, the MSCA noted that category 1B may be considered. Indeed, the MSCA pointed out that the duration of the monkey study might have been too short and the group of animals too low to observe an effect. Moreover differences in toxicokinetic cannot be ruled out. Finally, a MSCA highlighted that rat is considered a less sensitive species to effects on fertility and sperm than men and therefore that a more severe category need to be considered.

A late comment was received from an industry representative including new data. Industry argued that category 1B for fertility was not appropriate on the following basis:

- there are differences in metabolism between rats and monkeys, rats have similar but quantitatively more metabolite with less parent present;
- rodents are an insensitive model and a true fertility toxicant would also induce effects in other more sensitive species. Moreover, no effects were observed in male mice.

Furthermore, additional published and unpublished data were provided to give evidence of a difference in sensitivity in the tolerance of rat and human cells to flurochloridone levels and to give more insight to the flurochloridone mode of action. Two studies were published by Li *et al.* in 2018. In these studies, flurochloridone was shown to perturb rat Sertoli cell barrier function through Arp3-mediated F-actin disorganisation and increased apoptosis of Sertoli cells via ROS induction. Moreover, in a non-guideline unpublished non peer-reviewed study provided by the industry representatives (Frost, 2018), an increase in ATP and a decrease in glutathione were observed in rat Sertoli cell spheroids in presence or absence of rat S9. Decreased glutathione content was only observed in human Sertoli cells in the absence of rat S9. According to the

industry representative the data suggested that rat Sertoli cells are more sensitive to toxicity than human Sertoli cells and that metabolism reduced the toxic effects of flurochloridone.

Developmental toxicity

Two MSCA supported the proposal to classify flurochloridone as Repr. 1B, H360D.

Industry disagreed with the DS proposal to classify in category 1B and provided a position paper referring to Dorsal (2011). In their comment, they highlighted that flurochloridone was a developmental toxicant in rats but not in rabbits and that the highest dose used in the rabbit could not be considered too low as significant body weight loss was observed at GD6-12 with a reduction in food consumption in 4 animals (< 70 g/day vs 170 g/day in control). Moreover, the industry representative considered that maternal toxicity observed in developmental toxicity studies was under-predicted as similar general toxicity effects appeared only at dietary dose 10-fold higher than gavage dose. Thus, they concluded that a Cmax driven toxicological response occurred during the critical period of organogenesis was a likely explanation for the malformations observed.

Assessment and comparison with the classification criteria

Effects on fertility and sexual function

Available data provide clear and consistent evidence that flurochloridone altered spermatogenesis in the rat. Fertility was ultimately affected in male rats at ≥ 60 mg/kg bw/day in the 2-generation study and in the specific fertility study (Downs, 1983; Wilcynski, 1984). In these studies, other toxic effects mainly consisted of decreased body weight which was generally around 10-15%. Male reproductive organ weight and histopathology were consistently affected in the repeated-dose toxicity studies and in the 2-generation study. Effects in male reproductive organs in rats cannot be explained by the limited decreased in body weight and are not considered secondary to other toxic effects.

In the mechanistic study performed in male rats (Wilcynski and Killinger, 1985b), decreased sperm count in testis was observed since week 6 with change in spermatid release (decrease in stage 18 and 19 spermatids) and germ cell displacement since week 2 onward. Later, germ cell degeneration occurred along Sertoli cells vacuolisation.

Published studies from Li *et al.*, 2018 suggested that changes in sperm parameters might be induced by several factors. The authors suggested that they might firstly result from direct epididymal injuries caused by flurochloridone exposure and might also be caused by the loss of blood-testis barrier (Sertoli cell barrier) integrity which further disturbed testicular environment. In their *in vivo* study, sperm parameter changes were already seen after 2-weeks exposure in rats at around 30 mg/kg bw/day. At this dose level, no effects on weight was observed but testicular structure was injured with the loose of interconnection among some of Sertoli cells. In their *in vitro* studies, performed on 2D culture primary Sertoli cells, they showed that at non cytotoxic concentration (corresponding up to 300 mg/kg bw/day *in vivo*), abnormal changes in barrier permeability was observed (F-actin disorganisation and changes in the acting bindings protein expression of ARP3). Moreover, they also provided evidence of dose and time dependant relationship between flurochloridone exposure and apoptosis with the involvement of calcium intracellular disturbance and generation of ROS.

No effects on male reproductive organs were observed in repeated-dose toxicity studies in dogs and mice or in the fertility study in rabbits. Moreover, flurochloridone had no effects on spermatogenesis in the rabbits up to 33.9 mg/kg bw/day and in monkeys up to 64 mg/kg bw/day. RAC noted that in the rabbit, lower dose levels had been tested. In monkeys, sensitive endpoints were not investigated such as weight of sexual organs, histopathology and fertility.

It was commented during the public consultation that toxicokinetic in rats and monkeys might be different and that the duration of treatment in the monkey study might have been too short and group sizes too small to identify such effects in monkeys. There is no information in the dossier on potential kinetic differences between rats and monkeys.

Although some information of flurochloridone MOA were available (e.g. Sertoli cell disturbance), the precise mechanism of toxicity of flurochloridone on the male reproductive system is unknown. The relevance of the observed effects for humans were not challenged based on the available specific studies performed in male rats.

Moreover, the comparative *in vitro* study (Frost, 2018) is only considered as supportive data and of low weight. Indeed, the protocol used in the study was insufficiently described (e.g. strain of rat, number of human donors was not provided, no individual data). Moreover, cell viability in this 3D model was only partly investigated and the dose selection was not clearly justified in the report. The concentrations tested were up to 10 times that tested in the 2D rat Sertoli cell culture performed from Li *et al.*, 2018 showing cytotoxicity. Moreover, the results of the study were difficult to interpret as no changes either in rats or humans were statistically significant and for some parameters great variability and no dose-response was observed. Therefore, male rat Sertoli cells injuries observed were not sufficient to rule out effects in man in this *in vitro* study. In the rat cells, decreased glutathione content and increased ATP content were observed in the presence and absence of S9, oxidative stress and mitochondrial membrane potential increases were observed in the absence of S9 only. In human Sertoli cells (3D spheroid culture system), decrease in glutathione content was only observed in the absence of rat S9. Although Sertoli cells seems in this study more sensitive to flurochloridone toxicity than human Sertoli cells, glutathione disturbance was also observed in human cells. The suggestion that metabolism (rat S9) reduced the toxic effects of flurochloridone was not supported by *in vitro* data investigating genotoxicity.

While the available data provided clear evidence of male reproductive toxicity of flurochloridone in rats that is not secondary to other toxic effects, the absence of similar effects in mice, rabbits, dogs and monkeys might suggest a species-specific mode of action.

Nevertheless, uncertainties exist in relation to the toxicokinetics as no data were available to compare rats with mice, dogs and monkeys. Although no marked differences have been observed in the comparative toxicokinetic study in rabbits and rats, higher dose levels should have been used in the fertility study in male rabbits to rule out potential toxicity. Besides, it is not known whether the sensitivity of rats can be explained by mechanistic factors or toxicokinetic differences. On this basis, relevance to human cannot be excluded and a classification of flurochloridone for fertility is warranted.

In females, the ovary was also identified as a potential target organ. The marked dose-related increase in ovary weight at ≥ 31.4 mg/kg bw/day observed in the 90-day toxicity study in rats (but not in the multigenerational study) and the transient increase in ovary weight in the carcinogenicity study of mice at ≥ 32.1 mg/kg bw/day may be of concern. Moreover, in the developmental toxicity study in rats, malformation in testes but also in ovaries were observed at high dose (400 mg/kg bw/day) suggesting that ovary may also be a potential target organ. Therefore, effects observed in the ovaries are considered supportive for classification.

Overall, RAC concludes that clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects has been observed in rats. As there is no mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 1B for fertility is considered more appropriate than category 2.

Developmental toxicity

In rats the following effects were observed in the two prenatal developmental toxicity studies:

- Increased post-implantation losses at 400 mg/kg bw/day;
- Decreased foetal body weight, statistically significant from 100 mg/kg bw/day. This effect was reproduced in the second study at the same dose level;
- Increased incidence of external malformations: at 400 mg/kg bw/day foetal anasarca and omphalocele were increased and fell outside the range of historical control data.
- Visceral malformations were increased from 25 mg/kg bw/day and consisted mainly of diaphragmatic hernia, heart/great vessel anomalies. Although the incidence of diaphragmatic hernia was not statistically significant at 25 mg/kg bw/day, in view of dose-response, the effect was considered treatment related. No historical control data are presented for this effect. Other visceral malformations occurred only from 100 mg/kg bw/day (undescended testes, malpositioned incisors, malpositioned ovaries, retinal fold). Visceral variations were observed at ≥ 100 mg/kg bw/day (renal papilla/ureter effects) above historical control data. In the second study, the increased incidence of diaphragmatic hernia and heart/great vessel anomaly was reproduced and observed at 100 mg/kg bw/day. Visceral variations were also significantly increased at 100 mg/kg bw/day in the second study;
- Increased incidences of skeletal malformations and variations were seen at ≥ 100 mg/kg bw/day (vertebrae defect, used sternebrae, reduced ossification). These findings were also observed at 100 mg/kg bw/day in the second rat study.

With regards to maternal toxicity, in the first rat study (Nemec, 1983a) severe reduction in corrected body weight was observed at 400 mg/kg bw/day (33% of control) and food consumption was statistically significantly decreased at this dose level. At 100 mg/kg bw/day, only slight reduction in body weight gain could be observed and net bodyweight was only slightly decreased (87% of control). In the second rat study (Nemec, 1984a), net body weight gain was also only slightly reduced at 100 mg/kg bw/day and no effect on food consumption was observed.

In rabbits, slight embryotoxicity was observed at the highest dose of 60 mg/kg bw/day. According to the DS, this was mainly due to two dams with complete resorption, which was considered the consequence of maternal toxicity. Individual data are not available to support this statement. The main finding was the increase incidence of foetal skeletal variations and the slight delay in ossification observed at this dose level. In dams, at 60 mg/kg bw/day, clinical signs (reduced urinary and faecal output) were observed. Lower food consumption was observed during the second week of treatment and reduced body weight gain was noted during the first week of treatment. Nevertheless, no effects on corrected body weight was observed. RAC agrees with the DS that the tested dose levels in rabbit were not sufficiently high to rule out a teratogenic potential of flurochloridone in rabbits.

Overall, in view of clear evidence of teratogenicity in the rat studies, that could not be attributed to maternal toxicity, RAC agreed with the DS that classification in category 1B; for development is warranted.

Adverse effects on or via lactation

RAC agrees with the DS that there is no evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of milk and that no classification is warranted.

Overall, RAC considers that classification as **Repr. 1B; H360DF** is appropriate for flurochloridone.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

No classification is proposed by the DS as the substance is a solid and as no data in human indicated evidence of aspiration hazard.

Comments received during public consultation

No specific comments received.

Assessment and comparison with the classification criteria

RAC agrees with the DS that flurochloridone should not be classified as a substance that causes human aspiration toxicity hazard due to lack of data.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed that flurochloridone should be classified as Aquatic Acute 1; H400 (M-factor of 100) based on a 72h E_rC_{50} of 0.0047 mg/L for the alga *Scenedesmus [Desmodesmus] subspicatus*, and Aquatic Chronic 1; H410 (M-factor of 100) based on a 72h NOE_rC of 0.00028 mg/L for the same species and lack of rapid degradation. There are no long-term fish toxicity data, so the DS also considered the surrogate method for chronic classification, but used acute algal rather than the acute fish data.

Degradation

The substance is stable to hydrolysis with a half-life at 25 °C of > 1 year at pH 4, 7 and 9. It has a photolytic half-life of 15.9 – 18.1 days at a latitude of 50°N.

A GLP compliant ready biodegradation test according to OECD TG 301E (Modified OECD Screening test) showed no evidence of biodegradation after 28 days (based on dissolved organic carbon). Flurochloridone is not readily biodegradable.

Two aerobic simulation studies (German BBA Guideline Part IV, 5-1) both using two natural water-sediment systems indicated a low level of mineralisation (1.1 – 6.9%) after 100 days. The substance underwent rapid primary transformation, with a geometric mean whole system DT_{50} of 14.3 days at 20 °C (range 9.19 – 22.8 days) calculated for one study. Several transformation products were reported, but aside from indicating that the aquatic toxicity of two metabolites (R406639 and R42819, formed at ≥ 10% of applied radioactivity) is lower than that of the parent substance (based on data for algae and aquatic plants), the DS did not provide any further information on their hazard classification. The calculated whole system DT_{50} was 53.3 days for R406639 and 261 days for R42819.

According to the DS, flurochloridone does not undergo rapid abiotic degradation (the hydrolysis half-life is > 1 year at 25 °C at relevant pH), is not readily biodegradable and showed no evidence

of rapid mineralisation in simulation studies. They therefore did not consider it to be rapidly degradable.

Bioaccumulation

The octanol-water partition coefficient (log K_{ow}) is 3.36 at 25 °C (shake flask method). This value is below the CLP criterion of 4.

A flow-through fish bioconcentration study with Bluegill Sunfish *Lepomis macrochirus* gave a steady state bioconcentration factor (BCF) of 220 L/kg following a 28-day uptake period. The BCF is below the CLP criterion of 500 L/kg. The DS therefore did not consider flurochloridone to be bioaccumulative in aquatic organisms.

Aquatic toxicity

Aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold).

***Table 5**: Summary of relevant information on aquatic toxicity*

Method	**Test organism**	**Endpoint**	**Toxicity values in mg a.s./L**	**Reference**
Short-term toxicity to fish				
OECD TG 203 (flow-through)	*Oncorhynchus mykiss* (Rainbow Trout)	96h LC_{50}	3.0 (nominal)	Douglas *et al.*, 1987
US EPA-660/3-75-009 (static)	*Lepomis macrochirus* (Bluegill Sunfish)	96h LC_{50}	6.7 (nominal) [a]	Cohle & McAllister, 1983
OECD TG 204 (flow-through)	*O. mykiss*	28d LC_{50} 28d NOEC	1.87 (mean measured) 0.36 (mean measured)	Smith, 1990
Long-term toxicity to fish				
No data				
Short-term toxicity to aquatic invertebrates				
ASTM guideline (static)	*Daphnia magna*	48h EC_{50}	5.1 (nominal) [b]	Spare, 1983
Long-term toxicity to aquatic invertebrates				
OECD TG 211 (semi-static)	*Daphnia magna*	21d $NOEC_{repro}$	0.83 (mean measured)	Stewart *et al.*, 1990
Toxicity to algae and aquatic macrophytes				
US EPA Guideline 123-2 (static)	*Anabaena flos-aquae*	72h E_rC_{50} 72h NOE_rC	13.4 2.9 (mean measured) [c]	Wallace & Swarbrick, 2001
OECD TG 201 (static)	*Scenedesmus subspicatus*	72h E_rC_{50} 72h NOE_rC	**0.0047** **0.00028** (mean measured)	Bätscher, 2004a
OECD TG 221 and US EPA Guideline 123-2 (semi-static)	*Lemna gibba*	14d E_rC_{50} 14d NOE_rC	0.06 0.015 (mean measured)	Woodyer *et al.*, 2001

Note: a – Test concentrations were not measured, but the DS considered that as concentrations were well maintained in the other fish study, the nominal concentrations would be reliable.
b – No evidence is available about test concentration maintenance, but the DS considered that the concentration would be above 80 % of nominal after 48 hours without renewal based on data from the 21d *Daphnia* study.
c – Table 49 in the CLH report says nominal, but the text indicates mean measured.

Flurochloridone is a herbicide, and algae provide the most sensitive acute and chronic end points. RAC notes that the trans- isomer is three times more active as a herbicide than the cis- isomer according to the EFSA Draft Assessment Report, so different isomer ratios could affect the overall level of toxicity.

Some data are also available for the two transformation products observed in the water-sediment simulation studies above 10 % of applied radioactivity. R406639 has a 72h E_rC_{50} and NOE_rC of 3.3 and 1.0 mg/L (nominal), respectively, for *S. subspicatus*. R42819 has a 72h E_rC_{50} and NOE_rC of 2.3 and 0.3 mg/L (nominal), respectively, for *S. subspicatus*, and a 7d E_rC_{50} and NOE_rC of 8.2 and 0.46 mg/L (nominal), respectively, for *Lemna gibba*. No data are available for fish or *Daphnia* for either substance.

Comments received during public consultation

Four MSCAs provided public comments. One agreed with the proposed classification with no further comment. Two agreed but pointed out that the acute fish data should have been used for the surrogate approach rather than algae. The DS agreed. The third MSCA did not express an opinion about the classification but asked if 7d end points were available for the *Lemna* study. The DS said they were not. RAC notes that they would be unlikely to affect the hazard classification in this case.

Assessment and comparison with the classification criteria

Degradation

Flurochloridone does not undergo rapid abiotic degradation (the hydrolysis half-life is > 1 year at 25 °C at relevant pH; information on photolysis is not directly relevant to the degradation assessment due to light attenuation with depth and the presence of quenching agents, etc., in natural waters). It is not readily biodegradable and showed no evidence of rapid mineralisation in simulation studies; primary transformation can be rapid with a geometric mean whole system DT_{50} of less than 16 days calculated for one study, but at least one transformation product (R42819) would be classified as Aquatic Chronic 2 based on a long whole system half-life in a water-sediment system and a 72h algal NOE_rC in the range 0.1 – 1 mg/L. Classification information for other relevant transformation products is lacking. It is therefore not considered to be rapidly degradable according to the CLP Regulation.

Bioaccumulation

RAC does not consider the log K_{ow} measurement to be reliable because the method is not appropriate for a surface active substance (surface tension: 54.6 mN/m at 20 °C at 90% of the saturation concentration). The fish BCF of 220 L/kg cannot be lipid normalised in the absence of lipid data, but is below the CLP criterion of 500 L/kg. Flurochloridone is therefore not considered to be bioaccumulative in aquatic organisms.

Aquatic toxicity

Short-term aquatic toxicity data are available for three trophic levels. The lowest acute toxicity value is a 72h E_rC_{50} of 0.0047 mg/L for the alga *Scenedesmus [Desmodesmus] subspicatus*. As this is below 1 mg/L, the substance meets the criteria for classification with Aquatic Acute 1. As $0.001 < E_rC_{50} \leq 0.01$ mg/L, the M-factor is 100.

[RAC notes that there may be some uncertainty in the acute fish and *Daphnia* tests performed under static conditions, in which results were based on nominal concentrations without evidence of test concentration maintenance. The substance is unlikely to volatilise or degrade over the test periods but is moderately adsorptive (organic carbon-water partition coefficient: 490 – 1100) so a static test may experience some losses. Concentrations were 60 – 125% of nominal after 96 hours in the acute fish test performed under flow-through conditions. In the static algal studies, some test concentrations were 70 - 72% of nominal after 72 h. However, this does not affect the classification as algae are significantly more sensitive.]

Reliable long-term aquatic toxicity data are available for two trophic levels. The lowest long-term toxicity value is a 72h NOE_rC of 0.00028 mg/L for the alga *Scenedesmus [Desmodesmus] subspicatus*. As this is below 0.1 mg/L and the substance is not rapidly degradable, it meets the criteria for classification with Aquatic Chronic 1. As $0.0001 < NOE_rC \leq 0.001$ mg/L, the M-factor is 100.

No long-term toxicity data are available for fish. The acute 96h LC_{50} values for fish are in the 1 – 10 mg/L range. This would lead to a less stringent chronic classification (Aquatic Chronic 2) so does not affect the conclusion.

As the substance is a herbicide, data on other aquatic macrophytes (such as *Myriophyllum*) should be considered if they become available in future. In summary, RAC supports the DS' proposal to classify flurochloridone as **Aquatic Acute 1; H400** and **Aquatic Chronic 1; H410** with an **M-factor of 100 for both acute and chronic**.

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).