Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

flazasulfuron (ISO): 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea

EC Number: 600-514-0
CAS Number: 104040-78-0

CLH-O-0000007377-66-01/F

Adopted

30 November 2023

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ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLAZASULFURON (ISO); 1-(4,6-DIMETHOXYPYRIMIDIN-2-YL)-3-(3-TRIFLUOROMETHYL-2-PYRIDYLSULFONYL)UREA

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: flazasulfuron (ISO); 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea
EC number: 600-514-0
CAS number: 104040-78-0
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	1
Comment received				
No comment				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	Germany		MemberState	2
Comment received				
Since the substance has no corresponding EC entry, no EC name should be given in section 1.1 (table 1). ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment DE-CA Comments CLH-flazasulfuron_annex.docx				
Dossier Submitter's Response				
We agree with the comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2023	Netherlands		MemberState	3

Comment received

On the basis of the currently available information, we agree with the proposed classification as Aquatic Acute and Chronic 1.
However, more details on the methodology and findings sections of the L. gibba study by Anonymous (1996) (for which an EC10 of 0.000027 mg/L and EC50 of 0.000058 mg/L are reported) would improve the underpinning of the proposed classification. The reported endpoints are the lowest ecotoxicological effect values and, if deemed acceptable, would affect the M-factor(s). Currently, we feel there is not sufficient information presented to be able to conclude that this study is not acceptable for environmental classification. The DS is requested to provide details on at least the following points (if available):
- number of replications and number of plants per replicate;
- availability of a positive control;
- performance of a range-finding study;
- the actual biological results should be included in the result section;
- the ECx values are considered to be not reliable due to the statistical instability of the derivation. Still, the study also presents a NOEC. Why is this value not used instead of the EC10? Or is the NOEC also considered unreliable? Please mention this in the ODD.
- considering the instability of the test substance, the test should have been performed under flow-through conditions. Is it known why for a semi-static test has been chosen?

Specific comments
Page 153 and 165: The guideline mentioned for the photodegradation in water study of SL-160 is OECD 216. Please be aware that this is the Nitrogen Transformation Test for soil microorganisms. The correct guideline should be OECD TG 316.

Dossier Submitter's Response

The study report is not available. In the CLH report the information reported in the renewal Assessment Report, 2016 (RAR, Volume 3, Annex B9) has been considered and it was summarised.
In the RAR the following was stated:

- The test design included 7 replicates per test concentratiosn and in control.
- The test was started with 3 randomly selected 4-frond colonies per flask. "Colony" means an aggregate of mother and daughter fronds attached to each other. "Frond" means a single leaf-like structure.
- It was not mentioned whether a range finding study was performed.
- Due to uncertainties from the study, the NOEC value has also been considered unreliable.
- There is no explication on why a semi-static test have been chosen.

The biological results are:

Table 9.2.7/01-02: Summary of Total Number of Fronds per Test Flask at the Counting Dates

Conc. (µg/L)	**Parameter**	**Results**		
		72 hour/Day 3	**144 hour/Day 6**	**168 hour/Day 7**
Control	Mean	21.3	48.3	60.3
	SD	1.50	2.75	3.82
	m_d	0%	1.2%	1.0%
	N	7	7	7
0.010 (n.a.)	Mean	20.1	32.7	40.6
	SD	1.68	7.34	13.23

	m_d	0%	0.4%	1.8%
	N	7	7	7
0.032	Mean	21.6	46.7	57.7
(0.02)#	SD	2.64	6.07	10.19
	m_d	0%	0.3%	0.7%
	N	7	7	7
0.10	Mean	20.4	26.7	32.6
(0.04)#	SD	1.51	2.21	3.74
	m_d	0%	1.1%	5.5%
	N	7	7	7
0.32	Mean	15.1	17.7	19.0
(0.08)#	SD	2.79	3.45	3.70
	m_d	0%	4.3%	10.5%
	N	7	7	7
1.0	Mean	13.1	14.3	14.4
(0.31)#	SD	1.07	1.25	1.90
	m_d	0%	24.0%	40.8%
	N	7	7	7

 # = Mean measured test substance concentration
Mean = Arithmetic mean of total frond number
SD = Standard deviation of total frond number
m_d = % of mean number of dead fronds in relation to the total number of fronds in the test concentration
N = Number of replicate flasks tested

Table 9.2.7/01-3: Increase in Frond Number during the Test Period (ΔFN)

Concentration (µg/L	**Increase in Frond Number (ΔFN) and Percentage Inhibition**					
	0 - 72 hour		**0 - 144 hour**		**0 - 168 hour**	
	ΔFN	**%**	**ΔFN**	**%**	**ΔFN**	**%**
Control	9.3	na	36.3	na	48.3	na
0.010 (n.a.)	8.1	12.9	20.7*	43.0	28.6*	40.8
0.032 (0.02)#	9.6	-3.2	34.7	4.4	45.7	5.4
0.10 (0.04)#	8.4	9.7	14.7*	59.5	20.6*	57.3
0.32 (0.08)#	3.3*	64.5	7.1*	80.4	7.0*	85.5
1.0 (0.31)#	1.1*	88.2	2.3*	93.7	2.4*	95.0

**Mean value significantly lower than in control at the 0.05 level*
 # Mean measured test concentration
na: Not applicable
-negative value indicates an increase in growth relative to the control

Table 9.2.7/01-4: Growth Rates k (1/day) and Percentage Inhibition of k

Concentration (µg/L	**Growth Rate (k) and Percentage Inhibition**					
	0 - 72 hour		**0 - 144 hour**		**0 - 168 hour**	
	k	**%**	**k**	**%**	**k**	**%**
Control	0.19	na	0.23	na	0.23	na
0.010 (n.a.)	0.17	9.8	0.16*	29.3	0.17*	26.9
0.032 (0.02)#	0.19	-1.4	0.23	2.9	0.22	3.6
0.10 (0.04)#	0.18	7.2	0.13*	42.7	0.14*	38.4
0.32 (0.08)#	0.07*	61.9	0.06*	73.3	0.06*	72.6
1.0 (0.31)#	0.03*	84.5	0.03*	87.7	0.03*	89.0

** Mean value significantly lower than in control at the 0.05 level*
 # Mean measured test concentration
na: Not applicable
-negative value indicates an increase in growth relative to the control

Table 9.2.7/01-5: Dry Weight of Lemna Colonies (in mg/test flask) after 7 Days

Dry weight	**Nominal Concentration of the Test Substance (*µg/L*)**					
	Control	**0.010**	**0.032**	**0.10**	**0.32**	**1.0**

Mean ±SD N	21.9 2.8 7	7.0* 5.4 7	22.5 6.0 7	7.3* 2.9 7	1.0* 0.6 7	0.4* 0.4 7
% Inhibition compared to control	0.0	68.0	-2.7	66.7	95.4	98.2

mean = Arithmetic mean of dry weight
SD = Standard deviation
N = Number of replicate flasks tested

Specific comments:
It is a typo error. The guideline is OECD TG 316.

RAC's response

RAC noted that in the Dossier Submitter (DS) comments in the RAR it was stated that according to OECD TG 221, the doubling time of frond number in the control must be less than 2.5 days (60 hours), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d-1. The doubling time in the Anonymous 1996 study was 3.0 days and therefore the validity criteria were not met.

The DS also noted that the confidence intervals in the EC_x calculations were very wide and considered the calculations not reliable. NOEC could not be determined by the program but was determined by expert judgement.

The DS also noted that the growth of *Lemna gibba* was statistically different from the control after 6 and 7 days at the lowest tested nominal concentration of 0.010 µg/L. However, at the next nominal concentration tested, 0.032 µg/L, the growth rate was not statistically different from the control during the test period. The author considered that the reduced mean values of growth parameters at nominal 0.010 µg/L might be caused due to an irregular growth of the test plants at some of the test flasks after 6 or 7 days.

The DS also noted that the analytical results showed high variability even on freshly prepared test media (44-128 % of nominal). The aged samples with nominal concentrations of 0.32 and 1.0 µg/L showed mean recoveries of 9 % and 14 %, respectively. In the low-level samples the concentrations decreased below the determination limit of 0.005 µg/L. Also, reviewing the analytical results, a high background noise in the HPLC chromatograms were found. Several peaks of unknown compounds near or at the retention time of the test substance were detected.

RAC also noted that the pH during the test was 5.0-5.3 in contrast to the valid Anon. 1999 *Lemna gibba* test where the pH ranged from 7.4 to 8.9. It is mentioned in the CLH Report that the pKa of flazasulfuron is 4.37 ± 0.08 and water solubility is around 27 mg/L at pH 5, 2100 mg/L at pH 7 and at pH 9 the substance is not stable.

RAC agrees with the DS and is of the opinion that the test is not valid and not reliable.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	4
Comment received				
Was not reviewed				

Dossier Submitter's Response
Noted.
RAC's response
Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	5
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	Germany		MemberState	6
Comment received				
Although some uncertainty remains for numerical chromosomal aberrations (bone marrow exposure in the in vivo MN not clearly shown), we agree that classification is not required. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment DE-CA Comments CLH-flazasulfuron_annex.docx				
Dossier Submitter's Response				
Thanks for the support.				
RAC's response				
Thank you, you support for no classification is noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	7
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	Germany		MemberState	8
Comment received				
Contrary to the conclusion drawn by the DS, classification as Repr. 2 (H361d) based on the statistically significant and dose-related increase in ventricular septal defects (VSD) in the preliminary and main developmental toxicity study in Wistar-Imamichi rats (Anonymous 25 1988a and Anonymous 26 1988b) may be warranted.				

We agree with the DS (page 120) that "the interpretation of VSD data from Wistar-Imamichi strain is very difficult to interpret". However, we cannot support the conclusion that "far greater weight should be placed on the study in Sprague Dawley rats". The acceptable HCD (within the five-year time span period, mean 1.52 %) clearly show that the observed statistically significant increase at the highest dose level is above the mean and the upper range of the HCD, while control incidence was within the HCD. Additional HCD of Wistar-Imamichi rats, which were mentioned in the position paper by the applicant (Anonymous 35, 2019) also support this view and even strengthen the concern, as the mean incidence was reported to be 3.01% (and therefore below the incidence of 4 % at 300 mg/kg bw/d and 6.6 % at 1000 mg/kg bw/d).

An additional source of HCD is provide by Nakatsuka et al 1997 (Cong. Anom., 37: 47-138) who collected HCD from developmental and reproductive toxicity studies in Japan, which were performed between 1986 and 1993. In this publication, the mean incidence of VSD in Imamichi rats from Imamichi Institute for Animal Reproduction, Toxicology Research Center (the same laboratory in which the study with flazasulfuron was performed) was reported to be 1.49 % (range 0 - 3.5 %, 14 studies, 319 dams, 2150 foetuses examined, data). Therefore, a treatment-dependent effect of flazasulfuron on the VSD incidence seems likely, taking also into account the dose-response relationship. To better interpret the data, it would be beneficial if not only the mean and the range, but also the 95th percentile as well as the individual data of each study were presented (details about performing laboratory, strain of rats, year in which the studies were performed).

The DS further claims that VSD are "a transient alteration that tends to disappear during postnatal development" (page 126). However, this should not be used as an argument against classification, as this malformation is clearly adverse and it is not known whether the same effect in humans is reversible or not. This is also supported by the publication of Turner et al. 2002, which shows that VSD are not always reversible in children. The cohort study shows that, depending of the size of the defect, 0 % - 80 % of the VSD closed spontaneously (Turner et al. 2002). The authors of the study emphasise that VSD is a severe malformation. Further, we do not have any information about the size and location of the observed VSD in Wistar-Imamichi rats (muscular or perimembranous), which has a major impact on the reversibility.

The authors of the study "Temporal changes in incidence of VSD in Wistar-Imamichi foetal and breast-fed rats" (Anonymous 30 (1984)) claim that VSD are no longer present after birth. It is unclear whether it is technically feasible to perform transthoracic echocardiography in rat pups (to allow follow-up investigations of the same pups with the defects) or whether different pups were investigated at different time points. In any case, data in humans show that, depending on the size and location of the defect, surgery is needed, as the defects are not transient.

The DS further argues that "the incidence observed in teratogenicity studies in rats is so much lower than expected for a cardiovascular teratogen" (page 126). However, much lower incidences of ventricular septal defects caused by Azadirachtin (Neem) were considered sufficient to justify a classification into Repr. 2.

On page 103, the DS mentions, "the severity of maternal toxicity is not sufficient to be considered a direct cause of the following developmental effects". We agree with the DS that the observed maternal toxicity should not be considered causative for the observed VSD – which increases the concern. The applicant argues that "as a result of maternal

toxicity, fetal development could be delayed increasing the incidence of VSD". This is contradictory to Anonymous 32 (1997), which shows that intrauterine growth retardation is not necessarily associated with VSD.

A further argument for classification is the statistically significant increase in the incidence of extra ribs, clearly above of the HCD (see table in attached annex). Although we agree that the effect itself is not sufficient for classification, it may be considered supportive for a classification proposal .

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment DE-CA Comments CLH-flazasulfuron_annex.docx

Dossier Submitter's Response

Thanks for your comments. In our opinion flazasulfuron is a borderline case for developmental toxicity with reasonable doubts between classification as category 2 and no classification regarding the incidence of the relevant malformation ventricular septal defect (VSD) seen in Wistar Imamichi rats. The approach of DE represents a conservative approach different than the DS view but it could be perfectly plausible.

We would like to add some clarifications to some DE comments.

Data provided by DE coming from an additional source of HCD for VSD (Nakatsuka et al 1997; Cong. Anom., 37: 47-138) from the Imamichi Institute for Animal Reproduction, Toxicology Research Center (the same laboratory in which the study with flazasulfuron was performed) reported an incidence of 1.49% and a range of 0-3.5% in the period between 1986 and 1993. Since both pilot and main developmental studies with flazasulfuron were performed in 1983 with this substrain of Wistar rat, the DS is of the opinion that HCD provided in the period 1975-82 (1.47% and a range of 0-11.3%) and 1983-86 (1.52% and a range of 0-5.3%) are more appropriate for a comparison of the VSD incidence. We agree that more details on HCD would be useful for the discussion.

The following DS argument "the incidence observed in teratogenicity studies in rats is so much lower than expected for a cardiovascular teratogen" is referred to the comparison of the VSD incidence in Wistar Imamichi rats and Sprague-Dawley rats after treatment with the known teratogens ephedrine and trimethadione respectively. In line with the DE comment lower incidences that those observed with these known teratogens could lead to classification. From our understanding the key point for developmental toxicity for flazasulfuron is to conclude if the incidences observed in both pilot and main developmental studies with Imamichi rats are spontaneous or treatment related taking into account the whole avalaible data (HCD comparison, dose dependency, statistical significance, dose levels used, data for other developmental studies, specific data available for this substrain of Wistar rat and other additional considerations).

With respect to the incidence of extra ribs we don't share the DE view. The percentage of foetuses with a 14th extra rib was statistically significantly increased (11.2%) at 1000 mg/kg bw/day (15 vs 0 in controls). The incidence of this effect was higher than historical control data (HCD) for Wistar-Imamichi rats (mean foetal incidences of 0.68% and 0.61% for 1975-1982 and 1983-1986 periods, respectively). The presence of this extra rib (or supernumerary rib) is considered a rudimentary anomaly in rats, and is regarded to be of low toxicological and biological relevance, since they do not persist beyond post-natal day 40 to 60, and it is often associated with maternal stress. Consequently, this effect is not regarded relevant for classification.

RAC's response
Thank you for your comments. RAC concluded classification as Repr. 2; H361d is warranted based on the increased incidence of VSD in the PNDT study in Wistar-Imamichi rats.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	9
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	10
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	11
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	12
Comment received				
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
11.01.2023	France		MemberState	13
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	14
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	15
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	16
Comment received				
FR agrees with the proposal of classification for environmental hazards and with the proposed M factors (acute and chronic).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Ozone Layer

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	17
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2023	France		MemberState	18
Comment received				
Was not reviewed				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

CONFIDENTIAL ATTACHMENTS
1. DE-CA Comments CLH-flazasulfuron_annex.docx [Please refer to comment No. 2, 6, 8]