Committee for Risk Assessment

RAC

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

30 November 2023

CLH-O-0000007377-66-01/F

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

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted on **30 November 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical 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**

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

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

Administrative information on the opinion

Spain has submitted on **15 September 2022** 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 **14 November 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **13 January 2023**.

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 following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	016-085-00-2	flazasulfuron (ISO); 1-(4,6-dimethoxypyrimidin-2-yl)- 3-(3-trifluoromethyl-2-pyridylsulfonyl)urea	-	104040-78-0	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	016-085-00-2	flazasulfuron (ISO); 1-(4,6-dimethoxypyrimidin-2-yl)- 3-(3-trifluoromethyl-2-pyridylsulfonyl)urea	-	104040-78-0	**Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H400 H410	**Retain** GHS09 Wng	**Retain** H410		**Add** M = 1000 M = 100	
RAC opinion	016-085-00-2	flazasulfuron (ISO); 1-(4,6-dimethoxypyrimidin-2-yl)- 3-(3-trifluoromethyl-2-pyridylsulfonyl)urea	-	104040-78-0	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2 Repr. 2	**Retain** H400 H410 **Add** H373 (liver, muscle) H361d	**Retain** GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H373 (liver, muscle) H361d		**Add** M = 1000 M = 100	
Resulting Annex VI entry if agreed by COM	016-085-00-2	flazasulfuron (ISO); 1-(4,6-dimethoxypyrimidin-2-yl)- 3-(3-trifluoromethyl-2-pyridylsulfonyl)urea	-	104040-78-0	Repr. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H373 (liver, muscle) H400 H410	GHS08 GHS09 Wng	H361d H373 (liver, muscle) H410		M = 1000 M = 100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Pure flazasulfuron (purity 99.7%) is a solid with a melting point of 180 °C. A Differential Scanning Calorimetry test showed decomposition at 181.5 °C as evidenced by gas evolution and discoloration. Technical active ingredient (purity 97.4%) has a melting point of 147-150 °C.

Explosives

Flazasulfuron was not explosive in two A.14 tests. However, the A.14 test method is not equivalent to the test procedures referred to in the CLP regulation. The DS proposed no classification based on absence of functional groups associated with explosive properties.

Flammable solids

Flazasulfuron was not flammable in two A.10 tests. Accordingly, the DS proposed no classification.

Self-reactive substances

The DS noted that flazasulfuron is thermally unstable (decomposition at 181.5 °C) and contains S=O group, which may be associated with self-reactive properties according to Table A6.3 of UN-MTC. As there are no suitable data to evaluate this property, the DS proposed no classification due to lack of data.

Pyrophoric solids

The DS proposed no classification based on experience in handling.

Self-heating substances

Flazasulfuron was negative in an A.16 test. The DS pointed out that the substance is thermally unstable and that the A.16 test method is not equivalent to the UN N.4 test method referred to in the CLP regulation. The DS proposed no classification due to lack of data.

Substances which in contact with water emit flammable gases

The DS proposed no classification based on structure (no metals or metalloids) and experience in production and handling.

Oxidising solids

The dossier contains an expert statement (Reisinger, 2014) concluding that based on the structure of flazasulfuron and the oxygen balance, oxidising properties are not expected. The DS noted that the molecule contains oxygen atoms bound to sulphur (i.e. oxygen bonded not only to carbon or hydrogen) and considered that oxidising properties cannot be disregarded based on structure. They proposed no classification due to lack of data.

Organic peroxides

The hazard class is not applicable as flazasulfuron does not contain the peroxide group.

Corrosive to metals

The DS proposed no classification based on melting point above 55 °C.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC concurs with the DS's assessment and conclusions except self-heating substances and oxidising solids for which RAC assessment of these two hazards is provided below the agreed hazard classes:

- For explosives, no classification based on conclusive data is agreed;
- For flammable solids, no classification based on conclusive data is agreed;
- For self-reactive substances, no classification due to lack of data is agreed;
- For pyrophoric solids, no classification based on conclusive data is agreed;
- For substances which in contact with water emit flammable gases, no classification based on conclusive data is agreed;
- For organic peroxides, no classification based on conclusive data is agreed;
- For corrosive to metals, no classification based on conclusive data is agreed.

Self-heating substances

The phenomenon of self-heating generally applies only to solids. The surface of liquids is not large enough for reaction with air. The CLP criteria refer to a test temperature of 140 °C. Pure flazasulfuron (99.7%) has a melting point of 180 °C. Melting point of the technical active ingredient (97.4%), i.e. the form normally put on the market, is 147-150 °C. According to the Guidance on the application of the CLP criteria (CLP guidance), substances with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is greatly reduced. The self-heating potential of technical flazasulfuron will be prevented by melting just above the test temperature of 140 °C. Decomposition in liquid state, i.e. above the melting point, is addressed by other hazard classes such as self-reactive. Further, RAC is of the view that despite the differences between test methods A.16 and UN N.4, a negative result in an A.16 test may be considered conclusive under CLP. Therefore, RAC proposes no classification based on conclusive data instead of lack of data.

Oxidising solids

Oxygen and fluorine atoms in the flazasulfuron molecule are bonded only to carbon except the sulphonyl group, where the oxygen atoms are bonded to sulphur. However, sulphonyl group is generally not oxidising. RAC proposes no classification based on conclusive data instead of lack of data.

Conclusion

In summary, RAC proposes no classification due to lack of data for self-reactive properties, and no classification based on conclusive data for the remaining physical hazards.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The dossier contains two acute oral toxicity studies, one in rats and one in mice. No mortality was observed up to the top dose of 5000 mg/kg bw. Accordingly, the DS proposed no classification.

Acute dermal toxicity

The DS proposed no classification based on an acute dermal toxicity study in rats showing no mortality or clinical signs up to 2000 mg/kg bw.

Acute inhalation toxicity

The DS proposed no classification based on an acute inhalation toxicity study in rats reporting no mortality at 6.0 mg/L.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute oral toxicity

In the acute oral toxicity study in rats (Anonymous 11, 1988), 10 animals per sex and group received flazasulfuron in water at dose levels of 2500 or 5000 mg/kg bw. There were no mortalities or clinical signs of toxicity.

The acute oral toxicity study in mice (Anonymous 12, 1988) employing the same design as the rat study (dose levels, no. of animals) did not show any mortality or clinical signs either.

As the available oral LD_{50} values are greater than 2000 mg/kg bw, RAC agrees with the DS's proposal of **no classification**.

Acute dermal toxicity

In the acute dermal toxicity study in rats (Anonymous 13, 1988), flazasulfuron moistened with water was applied to the skin of 10 animals per sex and group at dose levels of 1000 or 2000 mg/kg bw for 24 hours. There were no mortalities, no clinical signs of toxicity and no gross findings at necropsy.

As the dermal LD_{50} is >2000 mg/kg bw, RAC agrees with the DS's proposal of **no classification**.

Acute inhalation toxicity

In the acute inhalation toxicity study in rats (Anonymous 14, 1988), 10 males and 10 females were exposed (whole-body) to flazasulfuron dust for 4 hours at a single exposure concentration of 6.0 mg/L (MMAD 4.0 µm, GSD 2.1). There were no mortalities and no gross abnormalities at necropsy at the end of the 14-day observation period. Clinical signs included chromodacryorrhea,

wetness around nose and mouth and reddish brown stains around the nose and mouth. All signs disappeared by the end of the observation period.

As the 4-hour LC_{50} is >5 mg/L, RAC agrees with the DS's proposal of **no classification**.

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

Summary of the Dossier Submitter's proposal

No effects were observed in the acute oral toxicity or neurotoxicity studies within the guidance value range for STOT SE 1 (≤ 300 mg/kg bw). The DS discussed a decrease in motor activity observed in the acute neurotoxicity study (Anonymous 37, 2002) at 1000 and 2000 mg/kg bw, but concluded that this effect was likely to represent general toxicity rather than a specific neurotoxic effect. Further, no relevant effects were observed after dermal or inhalation exposure. There were no signs related to respiratory tract irritation or narcotic effects. Consequently, the DS proposed no classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Acute neurotoxicity study in rats (Anonymous 37, 2002)

Flazasulfuron in aqueous methylcellulose was administered via gavage to SD rats (10/sex/group) as a single dose at 0, 50, 1000 or 2000 mg/kg bw. The post-dose observation period was 14 days. Neurobehavioural assessment took place on days 0 (5 hours post-dose), 7 and 14. Several parameters of motor activity were significantly altered from 1000 mg/kg bw 5 hours post exposure (e.g. reduced distance travelled, decreased ambulatory time, decreased total counts, slightly increased resting time). For details see "Supplemental information" in the Appendix. No other effects were noted on clinical observations or in functional tests.

Conclusion on classification

The available studies did not show any effects warranting classification in Category 1 or Category 2. Further, there was no evidence of respiratory irritation warranting classification in Category 3.

RAC discussed the decrease in motor activity observed in the rat acute neurotoxicity study. According to the CLP criteria for narcotic effects (CLP, Annex I, 3.8.2.2.2), *"narcotic effects in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia."* While RAC considered the decrease in motor activity as a treatment-related and adverse effect, it was concluded that this effect does not meet the CLP criteria for STOT SE 1, 2 or 3. Most importantly, it was noted that no clinical signs of toxicity were neither reported up to 5000 mg/kg bw in the acute oral toxicity studies in rats and mice (Anonymous 11 and 12, 1988), nor in the acute neurotoxicity study itself. Therefore, RAC concluded that **no classification is warranted for STOT SE,** in agreement with the DS.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on an *in vivo* skin irritation study in rabbits showing no irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

In the *in vivo* skin irritation study (Anonymous 15, 1993), flazasulfuron moistened with water was applied to the skin of 6 rabbits for 4 hours. No erythema, oedema or other dermal effects were observed in any of the animals during the 72-hour observation period.

In addition, no significant irritation was observed in the acute dermal toxicity study in rats (Anonymous 13, 1988) up to the highest dose tested of 2000 mg/kg bw or in a 21-day dermal study in rabbits (Anonymous 44, 1994) up to the highest dose tested of 1000 mg/kg bw/d.

RAC concluded that **no classification is warranted for skin corrosion/irritation**, in agreement with the DS.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification based on an *in vivo* eye irritation study in rabbits reporting slight irritation not meeting the classification criteria.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

In the *in vivo* eye irritation study (Anonymous 16, 1993), flazasulfuron was instilled into the eyes of 9 rabbits. In 3 animals the substance was washed out with water 2.5 min after instillation, which is not in line with OECD TG 405. The eyes of the remaining 6 animals remained unwashed. No iritis or corneal opacity were observed. The maximum individual mean scores (24, 48 and 72 h) for conjunctival redness and oedema in the unwashed eyes were 1 and 0.3 respectively. The effects were reversible within 72 hours.

Conjunctival redness or oedema in an animal study meet the criteria for classification if the mean score (24, 48 and 72 h) is ≥ 2 in at least 2 out of 3 tested animals. As the mean scores for conjunctival redness and oedema were below 2 in all animals, RAC concluded that **no classification is warranted for serious eye damage/irritation**, in agreement with the DS.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The dossier contains two studies, a Guinea pig maximisation test (GPMT) and a Buehler test, both are negative. The latter study was considered unacceptable by the DS because only a draft report was available. The DS proposed no classification based on the GPMT.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The GPMT (Anonymous 18, 1998) used 20 negative control and 20 treated female guinea pigs. Additionally, there were 10 positive control (DNCB) animals, and further 10 animals serving as negative control to DNCB. Intradermal induction concentration of 2.5% flazasulfuron caused irritation. Topical application was conducted with 50% flazasulfuron in white petrolatum and was preceded by SLS-pretreatment since no irritation was observed at this concentration in the pre-test. DNCB was applied at 0.1% for intradermal induction, 1% for topical induction, and 0.5% for challenge. Skin reactions were neither observed in any of the flazasulfuron-treated nor the corresponding negative control animals upon challenge. In the positive control group, all ten animals responded to challenge with severe irritation (score 3). In the negative control challenged with DNCB, 4 out of 10 animals showed a score of 1. The study is negative.

The Buehler test (Anonymous 17, 1995) was also negative. However, the available study report is only a draft without any signatures.

RAC concluded that, based on the GPMT, **no classification is warranted for skin sensitisation**, in agreement with the DS.

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

Summary of the Dossier Submitter's proposal

Repeated dose toxicity of flazasulfuron was investigated in rats, mice and dogs. According to the DS the main target organs were the liver and kidney. Effects within the guidance value (GV) range for classification were observed in male rats (kidney) and in dogs (liver). According to the DS, nephropathy in male rats was related to accumulation of α2u-globulin, which is a male rat-specific phenomenon. Severity of liver effects in dogs below the GV was not considered sufficient for classification. Therefore, the DS proposed no classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Kidney

Kidney effects within the (extrapolated) GV range were observed in a 2-year study in F344 rats (Anonymous 20, 1995) (testing doses of 0, 40, 400 and 2000/4000 (males/females) ppm, equivalent to 0, 1.313, 13.26 and 70.1 mg/kg bw/day for males and 0, 1.601, 16.45, and 172.6 mg/kg bw/day for females) at 400 ppm (13 mg/kg bw/d). Findings in males at the 1-year interim sacrifice included increased kidney weight (absolute by 16%), early change of chronic nephropathy (10/10 vs 3/10 in the control), increased hyaline droplets in proximal tubular cells (10/10 vs 0/10) and luminal dilatation of proximal tubules (10/10 vs 0/10). Histopathological examination of males from the 400 ppm group at the 2-year sacrifice showed increased incidence of chronic nephropathy and hyperplasia of pelvic epithelium. At 2000 ppm (70 mg/kg bw/d) all males died by week 93; the markedly increased mortality at the high dose was attributed to renal failure. There was no increase in kidney tumours. No kidney-related changes were observed at 400 ppm in females, but increased incidence of chronic nephropathy was observed in this sex at 4000 ppm (173 mg/kg bw/d). Further details can be found under "Supplemental information" in the Appendix.

In a follow-up mode of action (MoA) study (Anonymous 46, 1995), male F344 rats received flazasulfuron in corn oil via gavage for 2 weeks at dose levels of 0, 400 or 800 mg/kg bw/d. D-limonene was used as a positive control at 1500 mg/kg bw/d. Kidneys were subjected to histopathological examination (6 animals per group) and immunohistochemical examination for α2u-globulin (2 animals per group). Increased deposition of hyaline droplets in renal proximal tubular cells was observed in all animals at 400 mg/kg bw/d. These hyaline droplets in renal proximal tubules were positive for immunohistochemical staining with α2u-globulin. Similar findings were seen in the d-limonene group.

In a 90-day dietary study in F344 rats (Anonymous 41, 1988), no kidney effects were noted at 1000 ppm (57/62 mg/kg bw/d m/f). Findings at 5000 ppm (287/309 mg/kg bw/d m/f) included increased kidney weight (absolute by 52%/20% m/f) and in males focal tubular atrophy and dilation of proximal tubules.

A two-generation study in Sprague-Dawley rats (Anonymous 24, 1995) reported significantly increased incidence and increased severity of nephropathy in P and F1 males from 2000 ppm (ca. 140 mg/kg bw/d). A significant increase in the incidence of nephropathy was also seen in F1 females at 10000 ppm (ca. 800 mg/kg bw/d); P females were not examined.

The available information indicates involvement of α2u-globulin-mediated MoA in the development of renal damage in male rats. This MoA is specific to male rats. RAC notes that some renal effects were also observed in female rats, although only at higher doses, and the severity was lower than in males. While human relevance cannot be fully excluded, the involvement of α2u-globulin-associated response decreases the concern. RAC agrees with the DS that the kidney effects observed in male rats within the (extrapolated) GV range are not sufficient for a STOT RE classification.

Liver, skeletal muscle, thymus

Effects on the liver, skeletal muscle and thymus were observed in dogs. The 90-day and 1-year dog studies are summarised below.

<u>90-day oral study in dogs (Anonymous 42, 1994)</u>

Flazasulfuron in gelatine capsules was administered to beagle dogs (4/sex/group) for 13 weeks. The dose levels were 0, 2, 10, 50 and 250 mg/kg bw/d for males, and 0, 2, 10, 50 and 100 mg/kg bw/d for females. Haematological examination took place before the start of treatment and at

weeks 2, 4, 7 and 13. Clinical chemistry parameters were determined prior to treatment and at weeks 4, 7 and 13.

The top dose for males was 250 mg/kg bw/d. Top dose male no. 18 was killed in extremis at week 11 of treatment. This animal showed marked decreases in body weight and food consumption at weeks 10 and 11 and exhibited decreased spontaneous motor activity with no stool. On gross pathological examination this animal was found to have yellow-coloured tissues and hardened liver. Histopathological examination of the liver revealed severe hepatocellular degeneration/necrosis, severe inflammatory cell infiltration and severe bile duct proliferation. Clinical chemistry at week 7 showed increased ALT (alanine transaminase), AST (aspartate aminotransferase) and CPK (creatine phosphokinase).

The rest of top dose males (no. 17, 19, 20) did not show any treatment-related clinical signs. Body weight gain of animals 19 and 20 was markedly depressed (0.5 and 0.2 kg respectively; control mean 1.4 kg) and all top dose males experienced periods of body weight loss. Decreased erythrocyte and leukocyte count was found in animals 19 and 20 on haematological examination at week 13. Clinical chemistry examination at week 7 showed increased ALT, AST and CPK in all animals; mild increases in these parameters were also present at week 4, but no effect was seen at week 13. All top dose males surviving to study termination had enlarged livers and their skeletal muscles were pale in colour. Histopathological examination of the liver showed diffuse hepatocellular swelling, inflammatory cell infiltration and increased deposition of brown pigment in hepatocytes, Kupffer cells and histiocytic cells in the interstitium. The pigment was considered to be hemosiderin and lipofuscin (positive staining for Berlin blue and Schmorl reaction, negative for Hall method). Examination of skeletal muscles (femur, tongue, larynx) revealed atrophy/degeneration of moderate degree in all three animals. Histopathological findings in this group further included thymus atrophy and increased deposition of brown pigment in the spleen.

The top dose for females was 100 mg/kg bw/d. Body weight gain of females was unaffected and there were no treatment-related clinical signs. Clinical chemistry examination showed increased activities of ALT, AST and CPK at week 7. Histopathological changes in the liver included hepatocellular swelling and increased pigment deposition. Slight atrophy/degeneration of skeletal muscle was observed in 3 out of 4 females. Three animals also showed slight atrophy of the thymus. No histopathological findings in the liver, thymus or skeletal muscle were seen in control females.

Liver findings at 50 mg/kg bw/d included liver necrosis accompanied by increased ALT in both sexes (animals no. 15, 16 and 114). Mild increases in ALT were also observed in two males (no. 10, 11) at 10 mg/kg bw/d.

No effect on skeletal muscle was observed at ≤ 50 mg/kg bw/d.

Histopathological findings in the liver and skeletal muscle as well as plasma levels of selected enzymes at ≥ 10 mg/kg bw/d are summarised in the tables below. No histopathological findings were noted in these tissues at 0 and 2 mg/kg bw/d.

***Table**: 90-day study in dogs: histopathology of the liver and skeletal muscle in males*

Group / Animal no.	**Liver**						**Muscle**
10 mg/kg bw/d	**Swell.**	**Inflam.**	**Pigment**	**Necrosis**	**B.d.prol.**	**Gran.**	**Atrophy**
9		+	+			+	
10		+	+				
11		++	+			+	
12			+				
50 mg/kg bw/d	**Swell.**	**Inflam.**	**Pigment**	**Necrosis**	**B.d.prol.**	**Gran.**	**Atrophy**
13			+				
14			+				
15		+	++	+	+	+	
16		++	+	+	+	+	
250 mg/kg bw/d	**Swell.**	**Inflam.**	**Pigment**	**Necrosis**	**B.d.prol.**	**Gran.**	**Atrophy**
17	++		+				++
18[a]		+++	++	+++	+++		
19	++	+	+				++
20	+		+				++

Severity grades: +, slight; ++, moderate; +++, severe

Histopathological findings: Swell. = Hepatocellular swelling; Inflam. = Inflammatory cell infiltration; Pigment = Increased deposition of brown pigment; Necrosis = Hepatocellular degeneration/necrosis; B.d.prol. = Bile duct proliferation; Gran. = Microgranuloma; Atrophy = Skeletal muscle atrophy/degeneration

***Table**: 90-day study in dogs: selected clinical chemistry parameters in males*

Group / Animal no.	**ALT (U/l)**				**AST (U/l)**			
Control	**Week -1**	**Week 4**	**Week 7**	**Week 13**	**Week -1**	**Week 4**	**Week 7**	**Week 13**
1	14	12	16	15	15	21	18	24
2	15	21	23	25	16	19	18	30
3	21	23	24	28	24	24	25	26
4	14	16	20	19	15	19	21	23
mean	16	18	21	22	18	21	21	26
10 mg/kg bw/d								
9	17	17	24	28	16	17	18	22
10	16	22	54	18	16	21	23	22

11	11	19	41	42	18	24	27	27
12	21	20	25	23	25	26	24	27
mean	16	20	36	28	19	22	23	25
50 mg/kg bw/d								
13	16	12	19	16	27	24	41	22
14	18	18	28	27	13	20	23	25
15	17	79	58	91	18	31	23	26
16	13	13	37	275	18	18	24	39
mean	16	31	36	102	19	23	28	28
250 mg/kg bw/d								
17	10	25	602	21	18	30	965	31
18	16	18	727	-	20	26	1040	-
19	16	43	788	28	18	79	1037	20
20	14	23	606	26	18	22	1607	24
mean	14	27	681	25	19	39	1162	25
Group / Animal no.	**CPK (U/l)**				**ALP (U/l)**			
Control	**Week - 1**	**Week 4**	**Week 7**	**Week 13**	**Week - 1**	**Week 4**	**Week 7**	**Week 13**
1	116	121	111	116	253	219	200	178
2	172	103	118	130	249	201	214	196
3	183	130	170	116	196	198	162	145
4	155	201	144	135	202	206	180	134
mean	157	139	136	124	225	206	189	163
10 mg/kg bw/d								
9	122	137	142	142	189	185	157	116
10	153	199	146	141	216	196	209	204
11	114	136	100	93	260	191	186	176
12	201	174	146	106	207	191	154	125
mean	148	162	134	121	218	191	177	155
50 mg/kg bw/d								
13	287	117	424	108	278	274	229	186
14	127	162	219	172	238	218	211	178
15	117	125	108	94	243	249	247	336
16	106	92	107	99	269	228	255	1087

mean	159	124	215	118	257	242	236	447
250 mg/kg bw/d								
17	178	367	20320	276	285	270	428	387
18	175	377	14300	-	198	285	348	-
19	159	1659	21360	169	256	350	520	246
20	161	162	41640	126	161	207	307	299
mean	168	641	24405	190	225	278	401	311

***Table**: 90-day study in dogs: histopathology of the liver and skeletal muscle in females*

Group / Animal no.	**Liver**						**Muscle**	
10 mg/kg bw/d	**Swell.**	**Inflam.**	**Pigment**	**Necrosis**	**B.d.prol.**	**Gran.**	**Atrophy**	**Infilt.**
109								
110								
111								
112								
50 mg/kg bw/d	**Swell.**	**Inflam.**	**Pigment**	**Necrosis**	**B.d.prol.**	**Gran.**	**Atrophy**	**Infilt.**
113		+				+		
114		+	+	+		+		
115			+			+		
116			+					
100 mg/kg bw/d	**Swell.**	**Inflam.**	**Pigment**	**Necrosis**	**B.d.prol.**	**Gran.**	**Atrophy**	**Infilt.**
117	+	+					+	+
118	+		+					
119	+						+	
120	+	++	+	+	+	++	+	

Severity grades: +, slight; ++, moderate; +++, severe

Histopathological findings: Swell. = Hepatocellular swelling; Inflam. = Inflammatory cell infiltration; Pigment = Increased deposition of brown pigment; Necrosis = Hepatocellular degeneration/necrosis; B.d.prol. = Bile duct proliferation; Gran. = Microgranuloma; Atrophy = Skeletal muscle atrophy/degeneration; Infilt. = Mononuclear cell infiltration

***Table**: 90-day study in dogs: selected clinical chemistry parameters in females*

	ALT (U/l)				AST (U/l)				CPK (U/l)			
Control	**Week -1**	**Week 4**	**Week 7**	**Week 13**	**Week -1**	**Week 4**	**Week 7**	**Week 13**	**Week -1**	**Week 4**	**Week 7**	**Week 13**
101	22	27	26	26	23	25	21	24	167	163	135	145
102	18	17	18	18	24	25	23	23	186	159	125	91
103	15	15	17	16	20	22	24	23	120	128	131	114
104	20	22	21	22	29	26	25	21	304	202	167	178
mean	19	20	21	21	24	25	23	23	194	163	140	132
10 mg/kg bw/d												
109	14	17	17	20	13	21	19	19	156	166	141	139
110	21	21	21	21	20	25	22	23	129	204	158	150
111	16	20	18	21	20	18	20	20	138	124	116	89
112	23	23	23	25	20	21	22	22	128	99	99	78
mean	19	20	20	22	18	21	21	21	138	148	129	114
50 mg/kg bw/d												
113	13	16	16	18	18	17	21	20	105	90	95	70
114	18	21	24	103	17	19	21	30	124	130	161	114
115	17	22	26	26	24	35	38	32	172	521	378	194
116	23	22	22	24	30	30	35	33	164	135	111	110
mean	18	20	22	43	22	25	29	29	141	219	186	122
100 mg/kg bw/d												
117	27	27	30	28	23	23	50	26	163	138	439	127
118	24	25	23	24	26	19	20	18	150	97	101	83
119	18	19	131	28	18	21	153	19	142	168	2270	86
120	18	15	38	120	22	25	65	41	122	147	783	123
mean	22	22	56	50	22	22	72	26	144	138	898	105

Information on the incidence and severity of thymus atrophy is provided in the table below. Incidence of thymus atrophy was increased from 50 mg/kg bw/d, severity at or below the GV of 100 mg/kg bw/d was slight.

***Table**: 90-day study in dogs: thymus atrophy*

Males					
Dose (mg/kg bw/d)	**0**	**2**	**10**	**50**	**250**
No. of animals for histopathological examination	4	4	4	4	4
Thymus: atrophy	1 (1 +)	1 (1 +)	1 (1 +)	3 (3 +)	4 (2 +, 2 ++)

Thymus weights, absolute (g): mean [individual animal data]	7.6 [**5.0**; 7.7; 8.2; 9.4]	9.6 [**4.9**; 8.7; 11.5; 13.4]	5.8 [**3.4**; 4.9; 7.3; 7.4]	4.9 [**3.3**; **3.6**; **5.3**; 7.4]	2.7 [**2.0**; **2.8**; **3.4**; **8.6**]
Females					
Dose (mg/kg bw/d)	**0**	**2**	**10**	**50**	**100**
No. of animals for histopathological examination	4	4	4	4	4
Thymus: atrophy	0	0	0	1 (1 +)	3 (3 +)
Thymus weights, absolute (g): mean [individual animal data]	5.7 [4.8; 5.1; 5.4; 7.3]	6.5 [4.2; 6.4; 7.3; 7.9]	7.5 [3.8; 6.3; 6.8; 12.9]	5.9 [**3.8**; 5.5; 6.5; 7.8]	5.1 [**3.4**; **3.5**; **4.4**; 9.1]

Severity: +, slight; ++, moderate

Organ weights in bold: animals with a histopathological finding of thymus atrophy

1-year oral study in dogs (Anonymous 22, 1995)

The 1-year study was conducted in the same facility and used animals from the same source as the 90-day dog study. Flazasulfuron in gelatine capsules was administered to beagle dogs (4/sex/group) for 52 weeks. The dose levels were 0, 0.4, 2, 10 and 50 mg/kg bw/d for males, and 0, 2, 10 and 50 mg/kg bw/d for females. Clinical chemistry parameters were determined prior to treatment and at weeks 26 and 52.

There were no effects on skeletal muscle and no increases in CPK activity.

Two cases of thymus atrophy were noted in males: one at 10 mg/kg bw/d and one at 50 mg/kg bw/d; the severity was moderate and slight, respectively. No case of thymus atrophy was found in females.

Increased incidence of liver inflammation was observed in both sexes at 10 mg/kg bw/d (below the extrapolated GV). One male of this dose group (no. 13) showed inflammation of a severe degree, accompanied by increased ALT. Moderate inflammation with increased ALT was also seen in one male at 2 mg/kg bw/d.

Liver histopathology and ALT levels at ≥2 mg/kg bw/d are presented in the tables below.

***Table**: 1-year study in dogs: liver histopathology and ALT in males*

Group / Animal no.	**Liver histopathology**				**ALT (U/l)**		
Control	**Inflam.**	**Necrosis**	**B.d.prol.**	**Gran.**	**Week -1**	**Week 26**	**Week 52**
1					30	37	43
2				+	31	42	44
3					34	40	49
4					30	43	51
2 mg/kg bw/d	**Inflam.**	**Necrosis**	**B.d.prol.**	**Gran.**			
9					27	41	41
10					23	27	29
11	++				33	99	78
12					32	53	52

10 mg/kg bw/d	**Inflam.**	**Necrosis**	**B.d.prol.**	**Gran.**			
13	+++		++		34	141	45
14	+				42	45	51
15	+				28	32	29
16	+				39	54	52
50 mg/kg bw/d	**Inflam.**	**Necrosis**	**B.d.prol.**	**Gran.**			
17					27	33	35
18	+++				26	35	38
19	+++	++	++		31	294	248
20					40	45	42

Severity grades: +, slight; ++, moderate; +++, severe

Histopathological findings: Inflam. = Inflammatory cell infiltration; Necrosis = Hepatocellular necrosis; B.d.prol. = Bile duct proliferation; Gran. = Microgranuloma

0.4 mg/kg bw/d (males): no liver findings

***Table**: 1-year study in dogs: liver histopathology and ALT in females*

Group / Animal no.	**Liver histopathology**			**ALT (U/l)**			
Control	**Swell.**	**Inflam.**	**Lymp.h.**	**Week -1**	**Week 17**	**Week 26**	**Week 52**
101				26		34	37
102				40		43	43
103				29		40	41
104				28		35	68
2 mg/kg bw/d	**Swell.**	**Inflam.**	**Lymp.h.**				
105				73		50	54
106				32		47	43
107			++	30		37	34
108				29		37	39
10 mg/kg bw/d	**Swell.**	**Inflam.**	**Lymp.h.**				
109		+		35		42	43
110		+		29		37	39
111		+	++	32		47	42
112				28		30	30
50 mg/kg bw/d	**Swell.**	**Inflam.**	**Lymp.h.**				
113	++	++		28		31	31
114		++		25		41	38
115				26		37	37
116		+		33	175[a]	108	64

Histopathological findings: Swell. = Hepatocellular swelling; Inflam. = Inflammatory cell infiltration; Lymp.h. = Lymphoid hyperplasia around bile duct

[a] Unscheduled examination in an animal showing a transient decrease in food consumption at week 16

Classification for liver, skeletal muscle and thymus

- Liver: Hepatocellular necrosis (slight) accompanied by increased ALT was observed in 3 out of 8 animals at 50 mg/kg bw/d in the 90-day dog study. This effect is adverse and is considered to meet the classification criteria. Liver effects at lower dose levels, such as inflammation and mild elevations in ALT in individual males at 10 mg/kg bw/d in the 90-day study and at 2 mg/kg bw/d in the 1-year study, are not considered to meet the criteria for Category 1. Therefore, RAC is of the opinion that a classification in Category 2 for the liver is warranted.
- Skeletal muscle: Skeletal muscle atrophy/degeneration (slight) accompanied by distinct increases in creatine phosphokinase was observed in 3 out of 4 females at 100 mg/kg bw/d in the 90-day dog study. This finding is considered to represent a significant toxic effect meeting the classification criteria. Accordingly, RAC is of the opinion that a classification in Category 2 for skeletal muscle is warranted.
- Thymus: Increased incidence of thymus atrophy was observed in the 90-day dog study from 50 mg/kg bw/d. No clear effect was observed in the 1-year study up to 50 mg/kg bw/d. As the severity grade within the GV range for classification was 'slight' and the toxicological significance of the finding is unclear, RAC proposes no classification for the thymus.

Overall conclusion

RAC is concluded that a classification as **STOT RE 2; H373 (liver, muscle) is warranted.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The available *in vitro* mutagenicity assays and an *in vivo* micronucleus test were negative. Therefore, the DS proposed no classification.

Comments received during consultation

A member state competent authority (MSCA) supported the DS's proposal, noting that bone marrow exposure in the mouse micronucleus test was not clearly shown.

Assessment and comparison with the classification criteria

A complete *in vitro* mutagenicity test battery is available for flazasulfuron, all tests are negative (see the table below).

***In vitro* mutagenicity tests**			
Type of study; year	**Method**	**Result**	**Remarks**
Ames test 1987	Plate incorporation method Rat liver S9 Top concentration *S. typhimurium*	Negative ±S9 Cytotoxicity *S. typhimurium* from 100 µg/plate, *E. coli* at 5000 µg/plate	

	200 µg/plate, *E. coli* 5000 µg/plate Solvent water		
Mouse lymphoma assay 1993	Rat liver S9 Top concentration 500 µg/mL Solvent acetone	Negative ±S9 No or slight cytotoxicity Precipitation from 100 µg/mL	Top concentration selection based on solubility
Chromosomal aberration assay 1988	Chinese hamster lung cells Rat liver S9 Top concentration –S9 0.33 mM (130 µg/mL), +S9 10 mM (4100 µg/mL) Solvent water	Negative ±S9 Cytotoxicity –S9 (continuous treatment): mitotic index (MI) 53% of control at 0.33 mM Cytotoxicity +S9: MI 70% of control at 10 mM	−S9 only continuous treatment; short-term treatment –S9 not performed
Chromosomal aberration assay 2014	Chinese hamster lung cells Rat liver S9 Top concentration short-term treatment ±S9 4100 µg/mL (10 mM), continuous treatment –S9 1025 µg/mL Solvent physiological saline	Negative ±S9 Cytotoxicity in all experiments; continuous treatment –S9: relative cell growth 46% of control at 1025 µg/mL Precipitation from 513 µg/mL	

In the *in vivo* micronucleus test (Anonymous 19, 1995), 5 mice per sex, group and time of sacrifice received a single oral dose of flazasulfuron in aqueous carboxymethylcellulose at dose levels of 0, 1250, 2500 and 5000 mg/kg bw. Bone marrow cells were collected at 24 h, 48 h and 72 h after dose administration. No increase in micronuclei was observed in this study. There was no mortality or clinical signs of toxicity up to 5000 mg/kg bw/d. RAC notes that bone marrow exposure was not demonstrated in this study and can only be assumed based on indirect evidence (substance shown to be systemically available in rat ADME studies, systemic effects such as hepatocellular hypertrophy observed in mouse repeated dose studies). Despite this, there is no significant evidence about mutagenicity of flazasulfuron in view of the conclusively negative *in vitro* battery.

RAC agrees with the DS's proposal of **no classification for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two carcinogenicity studies are available, one in rats and one in mice. Neither of them showed neoplastic effects. Accordingly, the DS proposed no classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

2-year study in rats (Anonymous 20, 1995)

F344 rats (85/sex/group) received flazasulfuron in diet at dose levels of 0, 40, 400 and 2000 ppm for males and 0, 40, 400 and 4000 ppm for females. The top concentrations were equivalent to 70 and 173 mg/kg bw/d in males and females respectively. Top dose selection was based on a 90-day study (Anonymous 41, 1988). 50 animals per sex and group were used for the main group (104 weeks) and the remaining 35/sex/group for interim sacrifices at 26, 52 and 78 weeks (10 per sex, group and time point).

All top dose males were killed *in extremis* or found dead by week 93, a sharp increase in mortality occurred from week 75. The markedly increased mortality in top dose males was attributed to nephropathy. General toxicity in top dose females included reduced body weight (by 15% at termination), increased kidney weight and increased incidence of chronic nephropathy. No increase in neoplastic lesions was observed in this study.

18-month study in mice (Anonymous 21, 1995)

Crl:CD-1 (ICR) mice (60/sex/group) received flazasulfuron in diet at dose levels of 0, 500, 3500 and 7000 ppm for 78 weeks. The top concentration was equivalent to 987 mg/kg bw/d for males and 1170 mg/kg bw/d for females. Top dose selection was based on a 6-week study (Anonymous 38, 1992).

General toxicity at the top dose included slightly reduced body weight in females (by ca. 5%), increased liver weight (relative by 23%/33% m/f) and increased incidence of hepatocellular hypertrophy. There was no significant increase in neoplastic lesions.

Conclusion on classification

As the available carcinogenicity studies showed no neoplastic effects, RAC concluded **no classification is warranted for carcinogenicity**, in agreement with the DS's proposal.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The dataset of flazasulfuron for reproductive toxicity included a two-generation study in rats, one prenatal developmental toxicity (PNDT) study in rabbits and two PNDT studies in rats. The first rat PNDT study (Anonymous 26, 1988) was conducted in Wistar-Imamichi rats and showed an increase in ventricular septal defect. The second rat study (Anonymous 27, 1996) used Sprague-Dawley rats and did not show any increase in visceral malformations or variations. The PNDT study in rabbits and the two-generation study in rats did not show evidence of reproductive toxicity.

The DS discussed the ventricular septal defect (VSD) in rats and concluded that classification for developmental toxicity was not warranted mainly due to the following considerations:

- The Wistar-Imamichi strain of rats had a much higher background incidence of VSD compared to other rat strains.
- No VSD was observed in foetuses of flazasulfuron-treated Sprague-Dawley rats or NZW rabbits.
- There were studies indicating that VSD observed in Wistar-Imamichi or SD rats at the time of birth may close during the postnatal period.

Further, the DS proposed no classification for sexual function and fertility as well as for effects on or via lactation.

Comments received during consultation

1 MSCA commented on developmental toxicity and proposed classification in Category 2, putting forward the following arguments:

- The observed increase in VSD in Wistar-Imamichi rats was probably treatment-related.
- Although some reversibility of VSD was observed in rats, the malformation was adverse and VSD was not always reversible in children. Further, the size and location of the observed VSD in Wistar-Imamichi rats (muscular or perimembranous) was not specified, which had a major impact on reversibility.
- The increased incidence in extra ribs in the Wistar-Imamichi rat study, although not sufficient for classification on its own, provided additional support for classification.

In their response the DS admitted that the case could be considered as a borderline between no classification and Category 2. In their view the key point was whether the increase in VSD in the study in Wistar-Imamichi rats was related to treatment. As to the 14th rib, the DS stated that this anomaly was of low biological relevance since it did not persist beyond postnatal day 40-60 and was often associated with maternal stress.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

Range-finding one-generation study in rats (Anonymous 23, 1993)

Sprague-Dawley rats (16/sex/group) received flazasulfuron at dietary concentrations up to 10000 ppm (equivalent to 731/791 mg/kg bw/d m/f in the premating period). The pre-mating period lasted for 10 weeks, the study was terminated after lactation day 21. The range of investigated parameters was limited as this was a range-finding study. Top dose parents showed a body weight reduction (by 16%/12% m/f at the end of premating). Litter size was reduced (top dose 13.3 vs 16.5 in the control) but remained well within the historical control data (HCD) range (12.7-14.1; 3 studies within 3 years of the current study).

Two-generation study in rats (Anonymous 24, 1995)

In this two-generation study with one litter per generation, Sprague-Dawley rats (30/sex/group) received flazasulfuron at dietary concentrations of 0, 200, 2000 and 10000 ppm (top concentration equivalent to about 720/800 mg/kg bw/d m/f in the premating periods).

Parental animals showed reduced body weight and food consumption; the decrease in food consumption might have been partly related to low palatability. Body weight at the top dose was ca. 15% below the control value in the P generation (both sexes; females also during gestation and lactation), the decrease in the second generation was around 15% in females and 22% in males. Histopathological examination of the kidneys revealed tubular dilation and increased severity of nephropathy in P males and in both sexes of the F1 generation.

Reproductive parameters (such as mating index, fertility index and gestation length) and reproductive organ histopathology were unaffected. Reproductive organ weights, sperm parameters and puberty onset were not measured.

Repeated dose toxicity studies

No effects on reproductive organ weights or histopathology were observed in the available repeated dose studies in rats, mice and dogs.

Conclusion on classification

In the absence of effects related to fertility or sexual function in the available studies, RAC agrees with the DS's proposal of **no classification**.

Adverse effects on development

Range-finding PNDT study in Wistar-Imamichi rats (Anonymous 25, 1988)

Pregnant Wistar-Imamichi rats (8/group) were administered flazasulfuron via gavage from gestation day (GD) 6 to 15 at dose levels of 0, 50, 200, 500 and 1000 mg/kg bw/d. Top dose dams showed reduced food consumption (GD 6-15 by 27%) and a mild effect on body weight. Developmental findings at the top dose included slightly decreased foetal weight (by ca. 5%), an increase in post-implantation loss (19% vs 5% in the control) and 3 foetuses (out of 44) from 2 litters with ventricular septal defect (1 case in the control). 1000 mg/kg bw/d was selected as the top dose for the main study.

PNDT study in Wistar-Imamichi rats (Anonymous 26, 1988)

Pregnant Wistar-Imamichi rats were administered flazasulfuron in aqueous CMC via gavage from GD 6 to 15 at dose levels of 0, 100, 300 and 1000 mg/kg bw/d. The study was terminated on GD 21. Approximately half of the foetuses in each litter were examined for visceral abnormalities, the other half were processed for skeletal examination. Visceral abnormalities were examined according to the Wilson's technique except for the thoracic cavities. For examination of the thoracic cavities, a microdissection method described by Nishimura (1974, article in Japanese) was employed.

Maternal toxicity was observed only at 1000 mg/kg bw/d as reduced food consumption and body weight gain. Early resorptions were increased at the top dose but this effect appears to be partly related to maternal toxicity as the two animals with total litter loss (no. 72 and 78) had severely reduced food consumption (see "Supplemental information" in the Appendix for more details'). Foetal weight at 1000 mg/kg bw/d was decreased by 8% compared to control.

Visceral examination revealed an increase in ventricular septal defect (VSD) beginning at 300 mg/kg bw/d, a dose without marked maternal toxicity. The top dose foetal incidence of 6.6% exceeds not only the HCD mean of 1.5% but also the range of 0-5.3% (years 1983-86, the current study conducted in 1988). The mid-dose foetal incidence of 4.0% is within HCD range but probably related to treatment, given the obvious dose-response relationship. RAC notes that based on the concurrent and historical control data this abnormality was relatively frequent in this strain of rats.

Skeletal examination showed reduced ossification (metatarsals) and increased incidence of 14th rib (foetus-based incidence at the top dose 11%, no cases in concurrent control, HCD mean 0.6%, HCD range 0-3.1%).

***Table**: PNDT study in Wistar-Imamichi rats (Anonymous 26, 1988)*

Dose (mg/kg bw/d)	**0**	**100**	**300**	**1000**
Total no. of females	23	23	23	22[a]
Pregnant females	23	23	23	22
Total litter loss	0	0	0	2
No. of pregnant dams with live foetuses on GD 21	23	23	23	20
Food consumption GD 6-9 (g/day)	24	23	21*	15**
Food consumption GD 9-12 (g/day)	26	26	24	20**
Food consumption GD 12-15 (g/day)	29	28	28	23**
Body weight GD 6 (g)	268	269	270	268
Body weight GD 9 (g)	280	277	276	262**
Body weight GD 12 (g)	300	298	295	281**
Body weight GD 15 (g)	324	318	316	300**
Body weight GD 21 (g)[d]	400	394	396	370**
Post-implantation loss (%); (±SD)	5.5 (±5.2)	3.7 (±5.6)	4.7 (±5.5)	20.8[c] (±30.2)
Early resorptions (%); (±SD)[b]	4.4 (±5.3)	3.5 (±5.7)	2.9 (±5.0)	17.9[c] (±31.7)
Late resorptions (%); (±SD)[b]	1.1 (±3.2)	0.3 (±1.3)	1.7 (±4.0)	3.0 (±4.2)
Mean litter size	14.0	13.9	13.5	11.6
Foetal weight, males (g)	5.5	5.5	5.6	5.1**
Foetal weight, females (g)	5.2	5.2	5.2	4.7**
External examination: no. of foetuses	323	320	310	256
External abnormalities: foetuses (litters)	0	0	0	2 (2)[e]
Visceral examination: no. of foetuses	156	153	149	122
Interventricular septal defect: foetuses (litters), % of foetuses per litter[b]	2 (1) 1.1%	1 (1) 0.6%	6 (6) 3.7%	8 (7) 6.3%
Skeletal examination: no. of foetuses	167	167	161	134
14th rib: foetuses (litters), % of foetuses per litter[b]	0 0.0%	0 0.0%	2 (1) 1.1%	15 (5) 10.0%
5 ossified metatarsals in both hind limbs: % of foetuses[b]	99.4	94.3	96.5	72.8

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

[a] 1 top dose female showed marked weight loss before the start of dosing period and was excluded from the study without administration of the substance

[b] Statistical analysis not conducted / presented

[c] Without the two dams with total litter loss: post-implantation loss 12.9 (±16.7), early resorptions 9.6 (±18.0)

[d] Gravid uterus weight and corrected body weight not available in the study report

[e] One foetus absent lower vertebrae, vestigial tail and anal atresia. The other foetus absent lower vertebrae, tailless, anal atresia and clubfoot

PNDT study in Sprague-Dawley rats (Anonymous 27, 1996)

Mated CD-Crl:(SD)BR rats (24/group) were administered flazasulfuron in aqueous CMC via gavage from GD 6 to 15 at dose levels of 0, 100, 300 and 1000 mg/kg bw/d. The study was terminated on GD 20. All foetuses were examined for visceral abnormalities using the Staples' technique. In the Staples' technique, the beating heart of a decapitated foetus is examined after slitting the pulmonary artery from above the heart into the right ventricle. A functional septal defect is detected by observing blood spurting from the left into the right ventricle. Approximately half of the foetuses in each litter were processed for skeletal examination.

Top dose dams showed a mild reduction in food consumption (by 12% over the dosing period, followed by a compensatory increase). Corrected body weight was unaffected.

There was no increase in resorptions. Foetal weight was reduced by 17% at the top dose, no effect was seen at lower doses. Skeletal examination revealed reduced ossification. There was no increase in visceral malformations or variations. No ventricular septal defect was detected in any group (the total number of examined foetuses in this study was 1262). One top dose foetus had an abnormal course of the aortic arch.

PNDT study in rabbits (Anonymous 28, 1988)

Mated NZW rabbits (17/group) were administered flazasulfuron in aqueous CMC via gavage from GD 6 to 18 at dose levels of 0, 50, 150 and 450 mg/kg bw/d. The study was terminated on GD 28. All foetuses were examined for skeletal and visceral anomalies. Examination of hearts for the presence of ventricular septal defects was conducted after fixation according to the microdissection method described by Nishimura. Top dose selection was based on a preliminary study.

In the preliminary study, all 7 females at 1000 mg/kg bw/d stopped eating 2 days after initiation of dosing and did not resume until autopsy. One animal died and one was sacrificed moribund. Another female was sacrificed following abortion. Although the remaining 4 females survived to scheduled sacrifice on GD 28, no live foetuses were obtained. No adverse effects were observed in the preliminary study at the next lower dose of 300 mg/kg bw/d.

In the main study, 5 out of the 14 pregnant top dose (450 mg/kg bw/d) females were sacrificed between GD 20 and 23 due to abortion. In 4 of them the abortion was preceded by a period of 6 to 11 days when the animals consumed no or only very little food. The abortions in these four animals are considered secondary to general toxicity. No evidence of developmental toxicity was observed in this study. There were no heart abnormalities in any group.

Two-generation study in rats (Anonymous 24, 1995)

The study showed no effect on litter size, stillbirths or postnatal survival of pups. A decrease in pup weights was observed at the top dose of 10000 ppm (ca. 800 mg/kg bw/d) in both generations. Weights of top dose F1 pups were 0%, 10%, 15% and 18% below control on days 0, 7, 14 and 21 respectively. The decrease in F2 pups was 8%, 14%, 15% and 20% on PND 0, 7, 14 and 21 respectively. The effect on pup weight is considered to be at least partly related to concurrent maternal body weight, which was ca. 15% below control in both generations. Direct consumption of maternal diet by the pups is likely to have contributed to pup body weight reductions on PND 14 and 21.

Studies on reversibility of ventricular septal defect

The DS summarised in the CLH report (p. 88-94, 111-116) several publications listed by industry in support of their proposal of no classification.

Morita *et al.* (1984; in Japanese) mentioned an investigation of VSD incidence in foetuses of untreated Wistar-Imamichi rats (no. of animals not specified) from GD 20 to PND 14. Foetal incidence of VSD was 7.6% and 3.0% on GD 20 and 21 respectively. No cases of VSD were detected on PND 1 or later.

Solomon *et al.* (1997) studied alterations of the cardiac membranous ventricular septum in foetal, weanling (PND 21) and adult (5-10 months) Sprague-Dawley rats:

- Membranous VSDs were observed in 2.0% foetuses/litter (13/550 foetuses, 9/40 litters) on GD 21 but not in weanling (n = 188) or adult rats (n = 77). There was no case of a muscular VSD.
- Dietary restriction of dams (7 g of food per day, i.e. 25% of control, on GD 6-20) did not affect VSD incidence (ad libitum group 1.6%, diet-restricted group 1.3%).
- The authors noted that there is a high variability in published background incidences of VSD in Sprague-Dawley rats. They speculated that this variability may be partly due to different dissection techniques. Solomon *et al.* used modified Staples' technique, where the left ventricle was incised first, then pressure was applied to the right ventricle, and any patency was detected by blood streaming from the right ventricle to the left.

Fleeman *et al.* (2004) studied postnatal fate of trimethadione-induced VSD in Sprague-Dawley rats, with the following results:

- On GD 21, 7.6% (28/268) of examined foetuses from dams receiving 400 mg/kg bw/d trimethadione on GD 9 and 10 had membranous VSD, compared to 0.6% in the control. At 600 mg/kg bw/d the incidence of VSD was much higher (50%), the defects were larger and in part of the foetuses (10% of examined foetuses) associated with other vessel anomalies.
- Pup mortality between PND 0 and 4 in the postnatal study was 2.5%, 5.5% and 24% at 0, 400 and 600 mg/kg bw/d, respectively. Many of the examined dead pups in the trimethadione-treated groups had VSD.
- The incidence of VSD on PND 21 was 0.03%, 0% and 6.4% at 0, 400 and 600 mg/kg bw/d. These incidences have to be interpreted in view of the postnatal mortality. Still, they suggest that part of the VSDs may resolve postnatally.
- There was no correlation between foetal or pup weight and occurrence of VSD.

Turner *et al.* (2002) reported a cohort study in 290 children with isolated VSD. Out of the 227 cases of small VSDs, 144 (i.e. 63%) closed spontaneously and 3 were closed surgically. The authors concluded that they had found a high rate of spontaneous closure for small perimembranous and muscular defects, and also for moderate muscular defects.

Discussion of effects and conclusion on classification

The main developmental finding to be considered for classification is increased incidence of ventricular septal defect in Wistar-Imamichi rats. This increase is most likely treatment-related and began at a dose without marked maternal toxicity.

In rat embryos the interventricular septum normally closes by GD 16. If the septum fails to close, the defect allows the oxygen-rich blood from the left ventricle to flow into the right ventricle and into the lungs. Small VSDs may be asymptomatic and tend to spontaneously close postnatally, whereas larger defects may lead to mortality. Reports from standard PNDT studies often do not specify the size of the observed VSDs; this is also the case of the PNDT study with flazasulfuron in Wistar-Imamichi rats. Postnatal reversibility of VSD in Wistar-Imamichi rats has been

investigated in pups of untreated animals (Morita *et al.*, 1984) but not of flazasulfuron-treated animals.

RAC notes the marked differences in background incidence of VSD between rat studies. The reason for these difference is unknown but a genetic component and different techniques used to detect VSD may play a role.

A further developmental finding in Wistar-Imimamichi rats was increased incidence of 14th rib. A clear increase in extra ribs only occurred at the maternally toxic top dose of 1000 mg/kg bw/d. The second rat study, using Sprague-Dawley rats, reported a foetal weight reduction by 17% in the presence of mild maternal toxicity at 1000 mg/kg bw/d. The second rat study did not show any increase in malformations or variations besides decreased ossification. No evidence of developmental toxicity was seen in rabbits up to maternally toxic doses.

To sum up, increased incidence of ventricular septal defect was observed in flazasulfuron-treated Wistar-Imamichi rats in the absence of marked maternal toxicity. As there is no information on the size and postnatal reversibility of the defect, it is considered a malformation. A treatment-related increase in malformations observed in an animal study may lead to a classification in Category 1B.

However, in this case the concern about the VSD is decreased by the relatively high and variable background incidence in Wistar-Imamichi rats and lack of increase in heart anomalies in SD rats or NZW rabbits. Therefore, RAC concluded that a classification in **Category 2 for adverse effects on development is warranted**.

Effects on or via lactation

Pup weight reduction was observed in both generations of the two-generation study at the top dose of 10000 ppm (ca. 800 mg/kg bw/d). Pup weights on PNDs 7 and 14 were 10-15% below control. This effect is considered to be at least partly related to maternal toxicity (maternal bw during lactation approx. 15% below control in both generations). Pup weight on PND 14 may also have been affected by direct consumption of maternal diet. Information on presence of flazasulfuron or its metabolites in milk is not available. RAC agrees with the DS's proposal of **no classification**.

Overall conclusion on reproductive toxicity

RAC concluded that a **classification for adverse effects on development as Repr. 2; H361d** is warranted, mainly based on increased incidence of ventricular septal defect in rats. RAC also concluded **no classification for effects on sexual function and fertility and effects on or via lactation**.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

As flazasulfuron is a solid, the DS proposed no classification.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The substance is a solid with a melting point of ≥147 °C. RAC agrees with the DS's proposal of **no classification**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Flazasulfuron is currently classified in Annex VI Table 3 of the CLP Regulation with Aquatic Acute 1, H400 and Aquatic Chronic 1, H410. The Dossier Submitter (DS) proposed to confirm the existing aquatic classification and add M-factors to both categories. The DS proposed to classify flazasulfuron as Aquatic Acute 1 based on the E_rC_{50} of 0.0007 mg/L for *Lemna gibba* which warrants an M-factor of 1000 ($0.0001 < E_rC_{50} \leq 0.001$); Aquatic Chronic 1 is instead proposed based on the E_rC_{10} of 0.00028 mg/L for *Lemna gibba* which warrants an M-factor of 100 ($0.0001 < E_rC_{10} \leq 0.001$) for a non-rapidly degradable substance.

Degradation

There was one ready biodegradability test (OECD TG 301 B Manometry Respirometry Test) available for flazasulfuron. No degradation was observed in 28 days at pH 7.4 (Anon. 2014).

Flazasulfuron was not hydrolytically stable at pH 4, pH 5, pH 7 and pH 9 at 22 °C and 37 °C over a period of 30 days (Anon. 1995). The test guideline was unknown, but the DS considered the study acceptable. Kinetic evaluation of the raw data gave DT_{50} values of 3.44-3.86 days at pH 5 and 15.7-16 days at pH 7 (Anon. 2014). Metabolites DTPU, DTPP, TPSA and ADMP were detected. DTPU is the major product of hydrolysis at pH 4, pH 5 and pH 7. DTPP is the major product of hydrolysis at pH 9. TPSA and ADMP are minor products at pH 4 and 5.

In an aerobic mineralisation in surface water study (Anon. 2014, OECD TG 309), the half-lives for flazasulfuron were 21 and 25 days at concentrations 0.002 mg/L and 0.010 mg/L, respectively. Max. 0.6% mineralisation was detected after 90 days. Metabolites DTPU and DTPP were identified.

In a water/sediment study (Anon. 1996, BBA Guideline Part IV and others) the DT_{50} for the whole system was 23.9 days at pH 6.6 and at pH 7.7 (Anon. 2014). DTPU was the major degradation product. TPSA and HTPP were also detected.

No photolysis occurred in the two photochemical degradation studies performed under laboratory conditions (Anon. 2014, Anon. 1995). In water exposed to sunlight in the environment, the route of degradation of flazasulfuron was the initial hydrolysis to DTPU that accounted for about the 60 % of the initial flazasulfuron after 30 days. DTPU was slowly converted to DTPP.

The available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA, ADMP, HTPP) revealed toxicity values > 1 mg/L. Apart from these data, there is an acute toxicity test for metabolite SSRE-018 (GTPS) giving a 7-day E_rC_{10} (*Lemna gibba*) > 0.460 mg/L. Chronic data on fish and daphnia are lacking.

Based on the above, the DS considered flazasulfuron as non-rapidly degradable.

Bioaccumulation

Partition coefficient n-octanol/water study (Anon. 1994) was performed according to 40 CFR 158.190 method. Data at basic pH was not relevant because flazasulfuron is not stable under

alkaline conditions (e.g. pH 9). The substance is easily ionisable and will tend to remain in water at neutral or basic pH. The log K_{ow} values were < 0.06 at pH 7 and 1.30 at pH 5 at 25 °C indicating that flazasulfuron has a low potential to bioaccumulate.

No bioconcentration factor for fish was available.

Aquatic Toxicity

Reliable acute and chronic data are available for all three trophic levels including macrophytes. Major metabolites showed less toxicity than the parent substance and thus are not considered further for classification purposes.

Acute

***Table**: Reliable data on acute aquatic toxicity of flazasulfuron*

Method	Species	Test material	Results[1]	Reference
Fish				
US EPA FIFRA No. 72-1; ASTM Standard E 729 – 88, GLP Flow-through, pH 8.0-8.4	*Salmo gairdneri*	Flazasulfuron technical (97.1% purity)	96 h LC_{50} = 22 mg/L mm 102-127% of nom	Anonymous, (1995)
OK-US EPA FIFRA No. 72-1; ASTM Standard E 729 – 88, GLP Flow-through, pH 7.8-8.2	*Lepomis macrochirus*	Flazasulfuron technical (97.1% purity)	96 h LC_{50} > 98 mg/L mm 88-109% of nom.	Anonymous (1995)
Invertebrates				
US EPA 540/9-82-024, US EPA 540/9-85-006; ASTM E 729-88; FIFRA 72-2, GLP Flow-through, pH 7.7-8.1	*Daphnia magna*	Flazasulfuron technical (97.1% purity)	48 h EC_{50} > 106 mg/L mm 92-106% of nom. based on mean measured concentrations	Anonymous (1995)
Algae and aquatic plants				
OECD TG 201; US EPA OPPTS 850.5400 (1996), GLP static, pH 7.9-9.3	*Pseudokirchneriella subcapitata*	Flazasulfuron technical (98.96% purity).	72 h ErC_{50} = 0.0177 mg/L nominal (measured ±20% of nom.)	Anonymous (2011)
OPPTS 850.4400; OECD TG 22, GLP static, pH 7.4-8.9	*Lemna gibba*	Flazasulfuron technical (99.7% purity)	**7 d ErC_{50} = 0.0007 mg/L** (frond number) gmm 90-101% of nom. at start; < LoD to 66.9-88.1% at the end	Anonymous (1999)
OECD TG 239, GLP Static, pH 7.8-9.6	*Glyceria máxima* (Reed Mannagras)	Flazasulfuron technical (98.5% purity).	28 d ErC_{50} (shoot length)= 0.0242 mg/L mm start: 119% end: 19% of nom. sediment and pore water concentrations insignificant	Anonymous (2014)

The lowest acute toxicity result was a 7-day E_rC_{50} of 0.0007 mg/L (frond number, geometric mean measured concentration) for *Lemna gibba*. The study was conducted under static conditions for 7 days. Test plants were exposed in nutrient solutions with nominal test concentrations of 0.1, 0.2, 0.4, 0.8 and 1.6 µg/L. The Lemna colonies in each test flask were inspected for changes in frond number and appearance on day 2 and 5 and at the end of the test. The number of alive and dead fronds was counted. After the test termination the dry weight of all colonies was determined. No abnormalities were observed at the test plants at the concentration of up to and including nominal 0.4 µg/L. At nominal 0.8 and 1.6 µg/L the roots of several plants were shorter than in the control. The test concentrations were analytically verified. At the end of the test period, the lowest test concentration was below the determination limit and not analysed. The lowest test concentration is therefore given as initial measured concentration of 0.09 µg a.s./L. The other test concentrations were between 66.9 and 81.1 % of nominal at the end of the test period. For the re-evaluation, the geometric mean measured concentrations were used, corresponding to 0.16, 0.34, 0.66 and 1.42 µg a.s./L.

Chronic

***Table**: Reliable information on chronic aquatic toxicity of flazasulfuron*

Method	Species	Test material	Results[1]	Reference
Fish				
OECD TG 210; OPPTS 850.1400, GLP Flow-through, pH 6.8-8.1	*Oncorhynchus mykiss*	Flazasulfuron technical (96.7% purity)	89 d NOEC = 20 mg/L nom. (mm 83-99% of nom.)	Anonymous (2004)
Invertebrates				
OECD TG 211; OPPTS 850.1300; ASTM E 1193-97, GLP Semi-static, pH 8.1-8.5	*Daphnia magna*	Flazasulfuron technical (98.5% purity)	21 d NOEC (immobility) = 6.2 mg/L, mm: new: 99.8 - 111% old: 90.1 – 100% of nom.	Anonymous (2013)
OECD TG 202 (April 1984). Proposed updated version OECD 202 (June 1993). GLP Semi-static, pH 7.7-8.4	*Daphnia magna*	Flazasulfuron technical (95.1% purity)	21 d NOEC = 6.25 mg/L nom. (mm 77.3-120.3% of nom.)	Anonymous (1995)
Algae and aquatic plants				
OECD TG 201, US EPA OPPTS 850.5400 (1996), GLP Static, pH 7.9-9.3	*Pseudokirchneriella subcapitata*	Flazasulfuron technical (98.96% purity)	72 h NOE_rC = 0.0010 mg/L, 72 h E_rC_{10} = 0.0018 mg/L nom. (mm ± 20% of nom.)	Anonymous. (2011)
OPPTS 850.4400; OECD TG 221, GLP static, pH 7.4-8.9	*Lemna gibba*	Flazasulfuron technical (99.7% purity)	7 d NOE_rC (frond number) = 0.00034 mg/L **7 d E_rC_{10} (frond number) = 0.00028 mg/L** gmm 90-101% of nom. at start; < LoD to 66.9-88.1% at the end	Anonymous (1999)

Method	Species	Test material	Results[1]	Reference
OECD TG 239, GLP static, pH 7.8-9.6	*Glyceria maxima* (Red Mannagras)	Flazasulfuron technical (98.5% purity).	28 d NOE_rC = 0.0044 mg/L 28 d ErC_{10} = 0.00399 mg/L shoot length mm start: 119% end: 19% of nom. sediment and pore water concentrations insignificant	Anonymous (2014)

The lowest chronic toxicity value was a 7 d E_rC_{10} (frond number, geometric mean concentrations) of 0.00028 mg/L. The study is presented under Acute toxicity.

Comments received during consultation

One MS agreed with the proposed classification. Another MS agreed with the proposed classification as Aquatic Acute 1 and Chronic 1 but wanted more details on the methodology and findings on the *Lemna gibba* study by Anonymous 1996. The study reported an EC_{10} of 0.000027 mg/L and EC_{50} of 0.000058 mg/L which were considered to be not reliable due to the statistical instability of the derivation. The MS suggested to use the NOEC instead if reliable. The study was rated to be supplementary information by the DS.

The DS informed that the study report was not available. The information considered and summarised in the CLH Report is from the renewal Assessment Report, 2016 (RAR, Volume 3, Annex B9). The DS presented the key data in their answer. The test design included 7 replicates per test concentration and in the control. The test was started with 3 randomly selected 4-frond colonies per flask. It was not mentioned whether a range finding study was performed. In addition, there was no explanation on why a semi-static test had been chosen. Due to uncertainties in the study, the NOEC value has also been considered unreliable. The study was considered as supportive study also in the RAR.

RAC noted that in the Rapporteur Member State (RMS) 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 RMS also noted that the confidence intervals in the EC_x calculations were very wide (as shown in the result table below) and considered the calculations not reliable. NOEC could not be determined by the program but was determined by expert judgement. The RMS 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 RMS 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 detection 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.

The following results were provided in the RAR:

	Growth rate [frond number] [µg a.s./L]	Yield [frond number] [µg a.s./L]
EC_{10}	0.027 (0.017 – 0.043)	0.025 (0.014 – 0.042)
EC_{20}	0.035 (0.022 – 0.056)	0.030 (0.018 – 0.051)
EC_{50}	0.058 (0.032 – 0.102)	0.044 (0.023 – 0.082)
NOEC	0.02 [1]	0.02 [1]

Values in parenthesis: lower and upper 95 % confidence level
[1] The NOEC could not be determined by the program but was determined by expert judgment

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 pK_a 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.

Assessment and comparison with the classification criteria

Comparison with the criteria

Degradation

RAC agrees with the DS conclusion to consider flazasulfuron as non-rapidly degradable based on:

- No degradation in an OECD 301 B ready biodegradability test in 28 days.
- DT_{50} values for hydrolysis were 3.44-3.86 days at pH 5 and 15.7-16 days at pH 7. Metabolites DTPU, DTPP, TPSA and ADMP were detected. There is not enough data available to conclude on the aquatic classification of metabolites.
- DT_{50} of 21 and 25 days and 0.6 % mineralisation after 90 days were detected in the aerobic mineralisation in surface water study – no ultimate degradation.
- DT_{50} in the water/sediment study were 23.9 days for the whole system.

Bioaccumulation

RAC agrees with the DS conclusion to consider flazasulfuron having a low potential for bioaccumulation.

- The substance is easily ionisable and will tend to remain in water at neutral or basic pH. The log K_{ow} values were < 0.06 at pH 7 and 1.30 at pH 5.

Aquatic toxicity

- There are reliable acute and chronic toxicity data available for fish, invertebrates, algae and aquatic plants.
- The lowest acute toxicity result is a 7 d E_rC_{50} of 0.0007 mg/L (frond number) for *Lemna gibba*.
- The lowest chronic toxicity result is a 7 d E_rC_{10} of 0.00028 mg/L (frond number) for *Lemna gibba*.

RAC agrees with the DS proposal to classify flazasulfuron to **Aquatic Acute 1** based on the E_rC_{50} of 0.0007 mg/L for *Lemna gibba* (Annex I of CLP, Table 4.1.0 (a)) which **warrants an M-factor of 1000** ($0.0001 < E_rC_{50} \leq 0.001$) and to **Aquatic Chronic 1** based the E_rC_{10} of 0.00028 mg/L

for *Lemna gibba* (Annex I of CLP, Table 4.1.0 (b) (i)) which **warrants an M-factor of 100** ($0.0001 < E_rC_{10} \leq 0.001$) for a non-rapidly degradable substance.

RAC evaluation of hazardous to the ozone layer

Summary of the Dossier Submitter's proposal

The DS proposed no classification for hazardous to the ozone layer. The Ozone Depleting Potential (ODP) of flazasulfuron has not been measured because substance residues are unlikely to occur and persist in the atmosphere, due to the low volatility (vapor pressure: less than 1.33×10^{-5} Pa at 25ºC) and the rapid photochemical degradation in air of the active substance (the calculated (AOPWIN) half-life was 0.636 hours). Any accumulation of flazasulfuron in the troposphere is therefore unlikely to occur. Although no specific data was available, considering the chemical structure and other available information on the physico-chemical properties, the DS concluded that flazasulfuron was not expected to be hazardous to stratospheric ozone.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

A substance is considered hazardous to the ozone layer if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present danger to the structure and/or functioning of the stratospheric ozone layer (Regulation (EC) No 1272/2008, Annex I, 5.1.2.1).

Any substances having an ODP of greater than or equal to the lowest ODP (i.e. 0.005) of the substances currently listed in Annex I to Regulation EC No 1005/2009 should be classified as hazardous to the ozone layer (category 1).

There is no ODP or other data available indicating that flazasulfuron may present danger to the structure and/or functioning of the stratospheric ozone layer.

RAC agrees with the DS conclusion that, considering the chemical structure and other available information on the physico-chemical properties, flazasulfuron **does not meet the criteria for classification in this hazard class.**

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 and additional information (if applicable).

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

Appendix
Supplemental information - In depth analyses by RAC

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

Acute neurotoxicity study in rats (Anonymous 37, 2002): motor activity

Acute neurotoxicity study in rats: motor activity, males								
Dose (mg/kg bw)	**Distance traveled (cm)**	**Resting time (s)**	**Ambulatory time (s)**	**Stereotypic time (s)**	**Bursts of stereotypic (counts)**	**Horizontal counts**	**Total counts**	**Vertical count**
Baseline								
0	2629.2	429.5	129.0	341.5	1112.6	2145.5	1318.2	173.2
50	2379.3	476.5	114.6	309.0	984.9	1987.2	1234.6	144.1
1000	2491.0	450.4	121.8	327.8	1066.7	2056.9	1278.4	125.7
2000	2217.2	528.6	105.4	266.1	877.0	1728.9	1114.8	120.2
Day 0	++	++	++	++	++	++	++	++
0	2098.8	536.6	102.8	260.6	873.3	1584.1	1025.4	246.1
50	2073.2	535.3	100.9	263.8	867.2	1594.0	1040.0	212.7
1000	1024.7**	676.0*	51.2**	172.8**	517.7**	842.3**	496.9**	59.5**
2000	853.8**	726.5**	42.2**	131.3**	411.2**	649.7**	390.9**	55.9**
Day 7								
0	1479.3	559.6	52.1	288.3	615.2	1433.1	933.2	262.4
50	1792.9	499.8	62.1	338.1	719.1	1768.6	1189.1	353.7
1000	1920.6	489.9	70.0	340.2	776.9	1831.0	1241.9	266.3
2000	1630.3	522.7	57.8	319.5	685.2	1601.3	1053.0	278.2
Day 14								
0	2533.3	488.6	121.4	290.1	995.1	1828.9	1281.7	416.9
50	2819.3	477.3	129.7	293.0	1021.6	2071.9	1493.6	392.2
1000	2241.5	556.1	106.2	237.7	582.2	1560.1	105.7	251.0
2000	2257.8	541.1	106.6	252.3	878.5	1617.6	1132.0	278.4

++ Trend Significant at the 0.01 level; * Statistical significance at the 0.05 level; ** Statistical significance at the 0.01 level

Acute neurotoxicity study in rats: motor activity, females								
Dose (mg/kg bw)	**Distance traveled (cm)**	**Resting time (s)**	**Ambulatory time (s)**	**Stereotypic time (s)**	**Bursts of stereotypic (counts)**	**Horizontal counts**	**Total counts**	**Vertical count**
Baseline								
0	3415.4	381.3	156.2	362.6	1164.3	2796.1	1858.8	227.6
50	3220.3	405.7	105.3	344.0	1160.3	2495.7	1675.4	211.2
1000	3153.1	394.4	149.1	356.6	1174.1	2542.3	1669.1	227.5
2000	2886.8	404.2	139.1	356.8	1166.2	2422.3	1522.5	180.3
Day 0	++	++	++	++	++	++	++	++

Acute neurotoxicity study in rats: motor activity, females								
Dose (mg/kg bw)	**Distance traveled (cm)**	**Resting time (s)**	**Ambulatory time (s)**	**Stereotypic time (s)**	**Bursts of stereotypic (counts)**	**Horizontal counts**	**Total counts**	**Vertical count**
0	2456.9	519.9	112.6	267.5	877.9	1926.3	1305.2	279.1
50	2599.2	497.5	119.7	282.8	938.4	1988.5	1381.2	294.0
1000	1589.9	608.1	76.6	215.3	691.7	1233.0*	779.9*	124.3*
2000	1018.9**	666.5**	51.7**	181.8*	554.1*	850.0**	466.8**	56.4**
Day 7								
0	2913.0	497.1	128.6	274.3	928.6	2179.2	1596.0	465.7
50	2955.7	475.4	131.8	292.8	994.4	2305.9	1675.6	398.5
1000	3050.9	453.0	137.6	309.4	1056.4	2349.5	1705.4	503.8
2000	2586.9	490.0	119.1	290.9	967.1	2087.0	1463.0	390.1
Day 14								
0	3187.1	436.3	139.3	300.4	1020.3	2545.9	184.5	629.7
50	2944.4	470.4	129.8	299.8	980.4	2380.0	1702.4	41.7**
1000	3461.6	452.5	148.1	299.3	1032.3	2739.2	2066.5	647.3
2000	2637.9	478.4	120.3	289.2	951.5	2097.0	1480.5	424.9*

++ Trend Significant at the 0.01 level; * Statistical significance at the 0.05 level; ** Statistical significance at the 0.01 level

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

Kidney effects

2-year study in rats (Anonymous 20, 1995)

The test substance was administered via diet to F344 rats (85/sex/group). The top dose was 2000 ppm (70.1 mg/kg bw/day) in males and 4000 ppm (172.6 mg/kg bw/day) in females. 50 animals per sex and group were used for the main group (104 weeks) and the remaining 35/sex/group for interim sacrifices at 26, 52 and 78 weeks (10 per sex, group and time point). All top dose males were killed *in extremis* or found dead by week 93; a sharp increase in mortality occurred from week 75. The markedly increased mortality in top dose males was attributed to nephropathy. Kidney-related parameters in males and females are presented in the table below.

***Table**: 2-year study in rats: kidney-related parameters*

Dose in ppm (mg/kg bw/day) males/females	**0**	**40 (1.3/1.6)**	**400 (13/16)**	**2000/4000 (70/173)**
Males				
Dose (mg/kg bw/d)	0	1.3	13	70
Week 26 (interim kill)				
No. of animals examined	10	10	10	10
Body weight (g)	392	399	391	382
Kidney weight, absolute (g)	2.2	2.1	2.3	3.0**
Early change of chronic nephropathy	4	3	3	10**
Increased hyaline droplets in proximal tubular cells	0	0	7**	10**
Luminal dilatation of proximal tubules	0	0	0	10**
Creatinine (mg/dL)	0.97	0.95	1.00	0.96
Blood urea nitrogen (mg/dL)	16	16	16	16
Urine volume (mL/24 h)	7.9	8.3	8.0	10.2**
Urine specific gravity	1.069	1.065	1.073	1.063
Week 52 (interim kill)				
No. of animals examined	10	10	10	10
Body weight (g)	446	461	438	424
Kidney weight, absolute (g)	2.3	2.4	2.6**	5.2**
Kidney coarse surface (macroscopic observation)	0	0	0	10**
Kidney enlargement (macroscopic observation)	0	0	0	10**
Early change of chronic nephropathy	3	3	10**	0
Chronic nephropathy	0	0	0	10**
Increased hyaline droplets in proximal tubular cells	0	0	10**	10**

Luminal dilatation of proximal tubules	0	0	10**	10**
Increased brown pigment deposition in proximal tubular cells	0	0	0	4*
Creatinine (mg/dL)	0.87	0.87	0.86	1.13**
Blood urea nitrogen (mg/dL)	16	15	15	29**
Calcium (mg/dL)	10.1	10.2	10.1	10.3*
Phosphorus (mg/dL)	3.4	3.8	3.6	4.5**
Potassium (mEq/L)	3.26	3.22	3.28	3.75**
Urine volume (mL/24 h)	7.5	7.9	7.5	28.8**
Urine specific gravity	1.059	1.068	1.062	1.022**
Week 78 (interim kill)				
No. of animals examined	10	10	10	10
Body weight (g)	482	482	448	323**
Kidney weight, absolute (g)	2.5	2.6	3.1**	3.2**
Kidney: coarse surface (macroscopic observation)	0	0	0	10**
Kidney: enlargement (macroscopic observation)	0	0	10**	0
Early change of chronic nephropathy	8	8	6	0**
Chronic nephropathy	0	1	4*	10**
Increased hyaline droplets in proximal tubular cells	0	0	10**	10**
Luminal dilatation of proximal tubules	0	0	10**	10**
Increased brown pigment deposition in proximal tubular cells	0	0	2	10**
Pelvis epithelial hyperplasia	0	0	0	10**
Creatinine (mg/dL)	0.88	0.87	0.86	3.25**
Blood urea nitrogen (mg/dL)	15	14	14	109**
Calcium (mg/dL)	10.4	10.5	10.3	11.8**
Phosphorus (mg/dL)	3.3	3.1	3.3	8.0**
Potassium (mEq/L)	3.15	3.14	3.12	4.07**
Urine volume (mL/24 h)	7.8	7.8	9.5	36.5**
Urine specific gravity	1.062	1.068	1.060	1.015**
Main group (104 weeks)				
Total no. of animals in the main group	50	50	50	50
Alive week 104	44	36	39	0
No. of animals for histopathological examination	50	50	50	49

Kidney cyst (macroscopic observation)	0	0	0	6*
Kidney coarse surface (macroscopic observation)	1	2	14**	49**
Early change of chronic nephropathy	16	22	3**	0**
Chronic nephropathy	28	22	47**	49**
Increased hyaline droplets in proximal tubular cells	2	2	50**	49**
Luminal dilatation of proximal tubules	1	0	50**	49**
Pelvis epithelial hyperplasia	0	0	11**	41**
Increased brown pigment deposition in proximal tubular cells	0	3	18**	43**
Kidney mineralization	0	0	0	38**
Parathyroid hyperplasia[a]	0	0	0	42**
Femur fibrous osteodystrophy[b]	0	0	0	40**
Creatinine (mg/dL)[c]	0.95	0.97	1.04**	
Blood urea nitrogen (mg/dL)[c]	13	14	17**	
Calcium (mg/dL)[c]	10.3	10.5	10.4	
Phosphorus (mg/dL)[c]	3.2	3.4	3.5	
Potassium (mEq/L)[c]	3.24	3.23	3.36	
Urine volume (mL/24 h)[c]	7.8	8.8	12.5**	
Urine specific gravity[c]	1.057	1.057	1.052	
Females				
Dose (mg/kg bw/d)	0	1.6	16	173
Week 26 (interim kill)				
No. of animals examined	10	10	10	10
Body weight (g)	211	210	211	195**
Kidney weight, absolute (g)	1.24	1.23	1.25	1.26
Week 52 (interim kill)				
No. of animals examined	10	10	10	10
Body weight (g)	237	242	232	224
Kidney weight, absolute (g)	1.42	1.39	1.42	1.55**
Week 78 (interim kill)				
No. of animals examined	10	10	10	10
Body weight (g)	303	298	290	244**
Kidney weight, absolute (g)	1.67	1.63	1.68	1.73
Early change of chronic nephropathy	4	6	2	4
Luminal dilatation of proximal tubules	0	0	0	9**
Creatinine (mg/dL)	0.93	0.95	0.95	0.98

Blood urea nitrogen (mg/dL)	14	15	15	16*
Main group (104 weeks)				
Total no. of animals in the main group	50	50	50	50
Alive week 104	36	40	43	37
Body weight (g)[c]	314	322	311	271*
Kidney weight, absolute (g)[c]	1.75	1.90	1.88	1.99**
No. of animals for histopathological examination	50	50	50	50
Early change of chronic nephropathy	27	30	21	24
Chronic nephropathy	3	1	4	11*
Increased hyaline droplets in proximal tubular cells	0	1	0	0
Luminal dilatation of proximal tubules	0	0	0	49**
Increased brown pigment deposition in proximal tubular cells	2	2	1	29**
Creatinine (mg/dL)	1.10	1.06	1.04	1.06
Blood urea nitrogen (mg/dL)	13	16	13	14
Urine volume (mL/24 h)[c]	11.3	9.6	8.8	11.7
Urine specific gravity[c]	1.048	1.054	1.058	1.045

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

[a] No. of examined animals: 50, 14, 50 and 47 at 0, 40, 400 and 2000 ppm respectively

[b] No. of examined animals: 50, 13, 50 and 49 at 0, 40, 400 and 2000 ppm respectively

[c] 10 animals per group

RAC evaluation of reproductive toxicity

Maternal and developmental toxicity in the PNDT study in Wistar-Imamichi rats (Anonymous 26, 1988)

The following table shows selected parameters of maternal and developmental toxicity at the top dose of 1000 mg/kg bw/d.

Table: PNDT study in Wistar-Imamichi rats (Anonymous 26, 1988): individual data at 1000 mg/kg bw/d

Dam no.	Food consumption (g/day)			Bw gain (g)	Early resorptions (%)	No. of fetuses with VSD	No. of foetuses with external abnormalities
	GD 6-9	GD 9-12	GD 12-15	GD 6-9			
70	17	19	24	3	0	0	0
71	12	19	25	-13	0	0	0
72	1	19	25	-35	100	0	0
73	14	19	20	-3	0	1	0
74	13	22	26	-11	0	0	0
75	14	14	20	-10	0	1	0
76	18	24	28	8	0	0	0
77	10	15	18	-12	0	0	0
78	6	3	6	-29	100	0	0
79	22	28	28	8	0	0	0
80	17	23	24	-2	5	0	0
81	14	19	22	-2	0	1	1
82	18	23	23	-9	6	1	1
83	12	17	23	-20	75	0	0
84	17	20	24	-2	14	0	0
85	16	21	25	-5	6	1	0
86	22	25	27	2	0	0	0
87	20	24	27	3	8	0	0
89	16	22	23	-8	28	0	0
90	19	23	25	2	20	2	0
91	18	22	24	-5	30	0	0
92	17	19	24	4	0	1	0
Mean	15	20	23	-6	18		