Committee for Risk Assessment

RAC

Annex 1
Background document
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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It is based on the official CLH report submitted to consultation and additional information (if applicable).

Adopted

30 November 2023

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CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Chemical name: 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea; Flazasulfuron

CAS Number: **104040-78-0**

Index Number: **016-085-00-2**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FLAZASULFURON (ISO): 1-(4,6-DIMETHOXYPYRIMIDIN -2-YL)-3-(3-TRIFLUOROMETHYL-2-PYRIDYLSULFONYL)UREA

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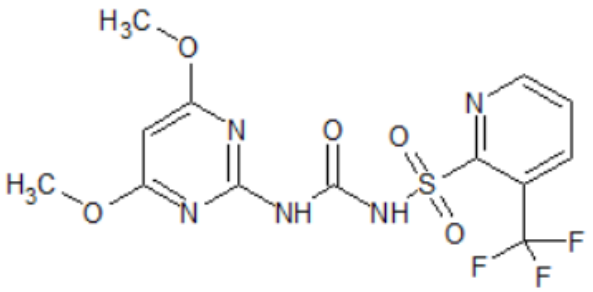
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea
Other names (usual name, trade name, abbreviation)	N-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(trifluoromethyl)-2-pyridinesulfonamide SL-160
ISO common name (if available and appropriate)	Flazasulfuron
EC name (if available and appropriate)	1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea
CAS number (if available)	104040-78-0
Molecular formula	$C_{13}H_{12}F_3N_5O_5S$
Structural formula	
SMILES notation (if available)	n1cccc(C(F)(F)F)c1S(=O)(=O)NC(=O)Nc2nc(OC)cc(OC)n2
Molecular weight or molecular weight range	407.36 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant – Flazasulfuron does not contain any stereoisomers
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant – Flazasulfuron is no UVCB
Degree of purity (%) (if relevant for the entry in Annex VI)	960 g/kg (96%)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**
1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-trifluoromethyl-2-pyridylsulfonyl)urea CAS No.: 104040-78-0	≥96% (w/w)	**Index No: 016-085-00-2** **Aquatic Acute 1, H400** **Aquatic Chronic 1, H410**	

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
According to dRAR (2016) there are no relevant impurities or additives in Flazasulfuron technical				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
According to dRAR (2016) there are no relevant impurities or additives in Flazasulfuron technical					

Table 5: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Not applicable				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: For substance with an existing entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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	**Modify** H410		**Add** M = 1000 M = 100	
Resulting Annex VI entry if agreed by RAC and COM	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		M = 1000 M = 100	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Not classified – conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Not classified – conclusive but not sufficient for classification	Yes
Self-reactive substances	Data lacking	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Not classified – conclusive but not sufficient for classification	Yes
Self-heating substances	Data lacking	Yes
Substances which in contact with water emit flammable gases	Not classified – conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data lacking	Yes
Organic peroxides	Hazard class not applicable	Yes
Corrosive to metals	Not classified – conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	Not classified – conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Not classified – conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Not classified – conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Not classified – conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Not classified – conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Not classified – conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Not classified – conclusive but not sufficient for classification	Yes
Carcinogenicity	Not classified – conclusive but not sufficient for classification	Yes
Reproductive toxicity	Not classified – conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Not classified – conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Not classified – conclusive but not sufficient for classification	Yes
Aspiration hazard	Not classified – conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	**Aquatic Acute 1; H400; M = 1000** **Aquatic Chronic 1; H410; M = 100**	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Data lacking	Yes

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Flazasulfuron is an active substance used as a herbicide in plant protection products (PPP).

Flazasulfuron is currently listed in Annex VI of Regulation (EC) 1272/2008. It has a harmonised classification as Aquatic Acute 1 (H400) and Aquatic Chronic, 1 (H410). This has been translated from the classification decided under the Dangerous Substances Directive 67/548/EEC (DSD) where it was classified as R50/53 by the European Chemicals Bureau (ECB) as presented in Commission Directive 2001/59/EC.

On 10 August 2016 the Authority (EFSA) communicated to the Commission its conclusion on whether flazasulfuron can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) 1107/2009. The Commission presented the renewal report for flazasulfuron to the Standing Committee on Plants, Animals, Food and Feed on 6 October 2016.

It has been established with respect to one or more representative uses of at least one plant protection product containing flazasulfuron that the approval criteria provided for in Article 4 of Regulation (EC) 1107/2009 are satisfied. It was therefore appropriate to renew the approval of flazasulfuron.

The risk assessment for the renewal of the approval of flazasulfuron was based on a limited number of representative uses, which however do not restrict the uses for which plant protection products containing flazasulfuron may be authorised. It was therefore appropriate not to maintain the restriction to use only as a herbicide.

Commission Implementing Regulation (EU) 2017/805 of 11 May 2017 renewed the approval of the active substance flazasulfuron in accordance with Regulation (EC)1107/2009 and amended the Annex to Commission Implementing Regulation (EU) No 540/2011.

Date of approval was 1 August 2017 and the expiration day was 31 July 2032.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Flazasulfuron is an active substance in the meaning of Regulation (EC) 1107/2009 (Article 36 of CLP Regulation).

5 IDENTIFIED USES

Flazasulfuron is an active substance used in plant protection products. It is used as a herbicide for the control of annual grasses and sedges.

Flazasulfuron is used as herbicide on vineyards, citrus and olive orchards (EFSA, 2016). The representative uses evaluated were spray applications in grapes, citrus and olives to control annual grasses and sedges.

6 DATA SOURCES

This CLH Report is mainly based on the available data from the draft Renewal Assessment Report (dRAR 2016) under development in accordance with Commission Regulation (EC) No. 844/2012 by the Spanish CA.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Comment (e.g. measured or estimated)	Reference
Physical state at 20°C and 101,3 kPa	Flazasulfuron (Batch No.Y-920205, pure active ingredient). Purity 99.7 % Odorless white solid powder (at 25ºC)	Visual assessment	Anonymous 47 (1996) (CA) B.2.3/01
	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.4 % Cream colour (Munsell colour 2.5Y 9/2), granular solid with a strong lawn fertiliser odour (at 24 ºC)	Visual assessment	Anonymous 48(1993) (CA) B.2.3/01
Melting/freezing point	Flazasulfuron (Batch No.Y-920205, pure active ingredient). Purity 99.7 % Melting point: 180 ºC	Method: EEC A.1 GLP: Yes	Anonymous 47 (1996) (CA) B.2.1/01
	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.4 % Melting point: 147-150 ºC	Method: metal block/capillary method (OECD 102) GLP: Yes	Anonymous 48 (1993) (CA) B.2.1/01
Boiling point	Flazasulfuron (Batch No.Y-920205, pure active ingredient). Purity 99.7 % Boiling point: Not determined due to decomposition at 181.5 ºC	Method: Differential Scanning Calorimetry (DSC) GLP: Yes	Anonymous 47 (1996) (CA) B.2.1/02
Relative density	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.4 % Relative Density D^{20}_{20}: 1.5958 and 1.6252. Average value: 1.6105	Method: EEC A.3 GLP: Yes	Anonymous 47 (1996)
Vapour pressure	Flazasulfuron (Batch No. 0201, Lot # Y.920205) Purity 99.8 % Vapour pressure at 25 ºC, 35 ºC and 45 ºC: < 1.33 x 10^{-5} Pa	Method: EPA OPPTS 830.7950 (Previously known as guideline 63-9) GLP: Yes	Anonymous 49 (1993) (CA) B.2.2/01
Surface tension	Flazasulfuron (Batch No. Y.920205) Purity 100 % 70.5 mN/m at 20 ºC (90% saturated aqueous solution)	Method: EEC A.5, OECD method 115 GLP: Yes	Anonymous 50 (2014) (CA) B.2.12/01

Property	Value	Comment (e.g. measured or estimated)	Reference
Water solubility	Flazasulfuron (Batch No. 0201, Lot # Y.920205) Purity 99.8 % Solubity at pH 5: 0.027 ± 0.003 g/L Solubility at pH 7: 2.100 ± 0.05 g/L Solubility at pH 9: not stable (T=25 ± 1 °C)	Method: EPA OPPTS 830.7860 (Previously known as guideline 63-8) GLP: Yes	Anonymous 51 (1994) (CA) B.2.5/01
Partition coefficient n-octanol/water	Flazasulfuron (Batch No.Y-920205, pure active ingredient). Purity 99.8 % Log Kow = 1.30 at 25ºC and pH 5 Log Kow < -0.06 at 25ºC and pH 7 Data not relevant at basic pH because Flazasulfuron (SL-160) is not stable under alkaline conditions (e.g., pH 9) under the study conditions used. Flazasulfuron is easily ionizable and will tend to remain in water at neutral or basic pH. The low mean KOW at pH 7 indicates that at this pH, flazasulfuron will not significantly partition into or tend to accumulate in living cells.	40 CFR 158.190 Guideline: 63-8 GLP: Yes	Anonymous 51 (1994) (CA) B.2.7/01
Flash point	Not applicable, melting point is > 40ºC.	-	
Flammability	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.3 % Not highly flammable	Method: EEC A.10 GLP: Yes	Anonymous 52 (1996) (CA) B.2.9/01
	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.3 % Non-flammable	Method: EEC A.10 GLP: Yes	Anonymous 47 (1996) (CA) B.2.9/01
Explosive properties	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.4 % Non-explosive	Method: EEC A.14 GLP: Yes	Anonymous 48 (1993) (CA) B.2.11/01
	Non-explosive	Method: EEC A.14, OECD Guideline 113 GLP: Yes	Anonymous 53 (1998) (CA) B.2.11/01
Self-ignition temperature	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.3 % No auto-inflammable	Method: EEC A.16 GLP: Yes	Anonymous 47 (1996) (CA) B.2.9/01

Property	**Value**	**Comment (e.g. measured or estimated)**	**Reference**
Oxidising properties	Non-oxidising	Statement	Anonymous 54 (2014) (CA) B.2.13/01
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	Flazasulfuron (Batch No.Y-920205, pure active ingredient). Purity 99.8 % At 25 ± 1 ºC: n-Hexane: 0.5 ± 0.04 mg/L Toluene: 0.56 ± 0.014 g/L Dichloromethane: 22.1 ± 0.54 g/L Methanol: 4.2 ± 0.10 g/L Acetone: 22.7 ± 0.75 g/L Ethyl acetate: 6.9 ± 0.21 g/L n-octanol: 0.20 ± 0.013 g/L Acetonitrile: 8.7 ± 0.18 g/L The active substance is soluble in all the organic solvents tested except hexane. No degradation was observed.	Method: EEC A.6, Flask method GLP: Yes	Anonymous 51 (1994) (CA) B.2.6/01
Dissociation constant	Flazasulfuron (Batch No. 0201, Lot # Y.920205) Purity 99.7 % pKa = 4.37 ± 0.08	Method: EPA OPPTS 830.7370 (Previously known as guideline 63-10) GLP: Yes	Anonymous 55 (1992) (CA) B.2.8/01
Viscosity	Not applicable, solid	-	

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14 GLP: Yes	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.4 % Non-explosive		Anonymous 48 (1993) (CA) B.2.11/01
EEC A.14 GLP: Yes	Non-explosive		Anonymous 53 (1998) (CA) B.2.11/01

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Flazasulfuron was tested for explosive properties using the EC Method A.14 and it was found not to be explosive.

8.1.2 Comparison with the CLP criteria

Flazasulfuron was not explosive according to test method EC A.14. However, this test method is not comparable to the test procedures for the classification of explosives described in Part I of the UN RTDG, Manual of Tests and Criteria.

Flazasulfuron does not contain any chemical groups associated with explosive properties as specified in Tables A6.1 in Appendix 6 of the UN Recommendations on Transport of Dangerous Goods (RTDG), Manual of Tests and Criteria. Therefore, flazasulfuron does not meet the criteria for classification as an explosive substance.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified – conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Table 10: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Hazard not applicable (solid)			

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Hazard not applicable (solid).

8.2.2 Comparison with the CLP criteria

Hazard not applicable (solid).

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard not applicable (solid).

8.3 Oxidising gases

Table 11: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Hazard not applicable (solid)			

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Hazard not applicable (solid).

8.3.2 Comparison with the CLP criteria

Hazard not applicable (solid).

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard not applicable (solid).

8.4 Gases under pressure

Table 12: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Hazard not applicable (solid)			

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Hazard not applicable (solid).

8.4.2 Comparison with the CLP criteria

Hazard not applicable (solid).

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard not applicable (solid).

8.5 Flammable liquids

Table 13: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Hazard not applicable (solid)			

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Hazard not applicable (solid).

8.5.2 Comparison with the CLP criteria

Hazard not applicable (solid).

8.5.3 Conclusion on classification and labelling for flammable liquids

Hazard not applicable (solid).

8.6 Flammable solids

Table 14: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10 GLP: Yes	Not highly flammable. Purity: 97.3% (Batch No.303, technical active ingredient)		Anonymous 52 (1996) (CA) B.2.9/01
EEC A.10 GLP: Yes	Not flammable. Purity: 97.3% (Batch No.303, technical active ingredient)		Anonymous 47 (1996) (CA) B.2.9/01

8.6.1 Short summary and overall relevance of the provided information on flammable solids

In an A.10 study, flazasulfuron did not ignite on contact with the ignition source.

8.6.2 Comparison with the CLP criteria

Flazasulfuron did not ignite on contact with the ignition source according to the method EC A.10, therefore, the criteria for classification as a flammable solid are not met.

8.6.3 Conclusion on classification and labelling for flammable solids

Not classified – conclusive but not sufficient for classification.

8.7 Self-reactive substances

Table 15: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
No data provided			

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No data available.

8.7.2 Comparison with the CLP criteria

A self-reactive substance corresponds to a thermally unstable solid liable to undergo a strongly exothermic decomposition even without participation of oxygen (air). Flazasulfuron has a melting point of 180ºC followed by thermal decomposition at 181.5ºC, thus thermally unstable.

Furthermore, flazasulfuron is a sulphonyl urea derivative and according to Table A6.2 of the UN Recommendations on Transport of Dangerous Goods (RTDG), Manual of Tests and Criteria, this functional group may be associated with self reactive properties. Therefore, no suitable data are available to evaluate this property.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification due to lack of data.

8.8 Pyrophoric liquids

Table 16: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Hazard not applicable (solid)			

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Hazard not applicable (solid).

8.8.2 Comparison with the CLP criteria

Hazard not applicable (solid).

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Hazard not applicable (solid).

8.9 Pyrophoric solids

Table 17: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
No data provided			

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No studies are available. However, flazasulfuron does not ignite spontaneously in contact with air based on experience of handling and use.

8.9.2 Comparison with the CLP criteria

According to *Section 2.10.4.1* of Annex 1 of CLP, the classification procedure for pyrophoric solids need not be applied when experience in manufacture and handling shows that the substance does not spontaneously ignite upon coming into contact with air at normal temperatures. There are no reports in the available studies of flazasulfuron spontaneously igniting when in contact with air.
Therefore, flazasulfuron does not meet the criteria for classification as a pyrophoric solid.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Not classified – conclusive but not sufficient for classification.

8.10 Self-heating substances

Table 18: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
Method: EEC A.16 GLP: Yes	Flazasulfuron (Batch No. 303, technical active ingredient). Purity 97.3 % No auto-inflammable		Anonymous 47 (1996) (CA) B.2.9/01

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

Flazasulfuron is not an auto-inflammable substance when tested for auto-flammability using the method EC A.16.

8.10.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the test method A.16 is not deemed appropriate to evaluate the self-heating property of solids towards a CLP classification.

Furthermore, Flazasulfuron is thermally unstable having a melting point of 180ºC and thermal decomposition at 181.5ºC. If self-heating behaviour cannot be ruled out by a screening test, further testing becomes necessary using UN Test N.4 in Part III, *Sub-section 33.3.1.6* of the UN-Manual of Tests and Criteria. Therefore, no suitable data are available to evaluate this property.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification due to lack of data.

8.11 Substances which in contact with water emit flammable gases

Table 19: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
No data provided			

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No data available.

8.11.2 Comparison with the CLP criteria

According to *Section 2.12.4.1* of Annex I of CLP, the classification procedure for this hazard class need not be applied if the chemical structure of the substance or mixture does not contain metals or metalloids, or experience in production or handling shows that the substance does not react with water or the substance is known to be soluble in water to form a stable mixture. According to the mentioned criteria classification for this hazard class is not applicable to flazasulfuron.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified – conclusive but not sufficient for classification.

8.12 Oxidising liquids

Table 20: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Hazard not applicable (solid)			

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Hazard not applicable (solid).

8.12.2 Comparison with the CLP criteria

Hazard not applicable (solid).

8.12.3 Conclusion on classification and labelling for oxidising liquids

Hazard not applicable (solid).

8.13 Oxidising solids

Table 21: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Statement	Non-oxidising		Anonymous 54 (2014) (CA) B.2.13/01

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

Flazasulfuron has two electronegative atoms: oxygen and fluorine. The oxygen is bound to either carbon (oxidising properties are unlikely) or sulphur, which has a similar electronegativity as carbon (2.58 to 2.5). The fluorine atoms are bound to carbon only. In both cases (oxygen and fluorine) oxidising properties are unlikely. Considering this, oxidising properties of Flazasulfuron are not expected by consideration of the structural formula.

8.13.2 Comparison with the CLP criteria

According to *Section 2.14.4.1* point b) of Annex I of CLP, for organic substances the classification procedure for this hazard class shall not apply if the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen. Flazasulfuron contains fluorine and oxygen atoms although oxygen is bound to sulphur. Therefore, this property cannot be disregarded based on chemical features of the molecule.

8.13.3 Conclusion on classification and labelling for oxidising solids

No classification due to lack of data.

8.14 Organic peroxides

Table 22: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
No data available			

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Flazasulfuron is not an organic peroxide. It does not contain the bivalent O-O functional group.

8.14.2 Comparison with the CLP criteria

Hazard class not applicable.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable.

8.15 Corrosive to metals

Table 23: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
No data provided			

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data derived in accordance with the recommended test method in CLP (test in Part III, *sub-section 37.4* of the UNRTDG Manual of Tests and Criteria) have been provided.

8.15.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (version 5.0 July 2017), the UN Test C.1 excludes solids while it considers 'solids that may become liquid upon transportation'. Flazasulfuron is supplied as a dry solid and its measured melting point is > 55ºC, which is the test temperature required in the UN Test C.1 test. Furthermore, evidence from manufacture and handling shows that flazasulfuron is not corrosive to metals. Therefore, flazasulfuron does not meet the criteria for classification as corrosive to metals.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – conclusive but not sufficient for classification.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 24: Summary table of toxicokinetic studies

Type of study/guideline, deviation if any/species/route/dosage/test substance	Results/Remarks	Reference
***IN VIVO* SINGLE DOSE**		
Distribution and excretion EPA Guideline 85-1. Comparable to OECD TG 417 (2010) GLP: Yes Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8% [^{14}C]- SL-160 (P) (pyridinyl ring), purity 97.5%, Lot No. CP-1385 **Species**: Rat, Sprague Dawley (♂,♀) **Vehicle**: Aqueous 0.75 % (w/v) methylcellulose **Route/Dose**: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (volume 10 ml/kg) **Observation period**: 7 days **Pilot study**: Not CO_2 or volatile organics were expired during pilot study. So expired air is not collected. **No. of animals**: 5 ♂ and 5 ♀ per dose level (2 rats/sex were used as control) **Radiactivity per animal**: 55 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times **Study acceptable**	Recovery of administered dose (AD) Recoveries at the end of the study were 100 % or over. | Dose Level / Sex	Urine %AD	Cagewash %AD	Feces %AD	Tissues %AD	Total %AD
---	---	---	---	---	---
Low Male	74.51 ±4.78	1.06 ±0.46	21.13 ±3.60	3.90 ±2.05	100.61 ±2.23
Low Female	93.18 ±2.54	1.21 ±0.45	10.13 ±0.94	0.98 ±0.57	105.49 ±2.15
High Male	79.92 ±2.95	0.34 ±0.12	23.66 ±1.73	1.33 ±0.22	105.25 ±1.68
High Female	93.78 ±7.06	0.67 ±0.28	9.21 ±1.31	0.48 ±0.06	104.14 ±6.88 | Distribution After 7 days, very low radioactivity recovered in tissues. Similar pattern was observed in both dose level groups although overall females seem to have a different distribution than males, which suggests differences in metabolism. **Low dose**: Total radioactivity in tissues was lower in ♀ than ♂. The highest concentration of radioactivity was found in blood in both sexes (0.99% AD in ♂ and 0.24 % AD in ♀). Carcass and muscle radioactivity was also high (2.51% and 0.85% AD, respectively in ♂ and 0.62% and 0.13%, respectively in ♀). **High dose**: Total radioactivity in tissues was lower in ♀ than ♂. The highest concentration of radioactivity was found in blood in both sexes (0.40% AD in ♂ and 0.14 % AD in ♀). Excretion Most of the radiolabelled compound was excreted at 48 h post-dosing except for low dose male group. The primary route of excretion was *via* urine and a minour route through faeces. The total excretion was almost completed 7 days after the administration. Similar pattern was observed in both dose level groups although significant differences between sexes, which suggests females have rapid excretion. **Low dose**: Urine was the main route of excretion in both sexes although at different percentages (75 % AD in ♂ and 93 % AD in ♀). Excretion in faeces accounted for 21 % AD in ♂ and 10 % AD in ♀). **High dose**: Urine was the main route of excretion in both sexes although at different percentages (80% AD in ♂ and 94 % AD in ♀). Excretion in faeces accounted for 24 % AD in ♂ and 9 % AD in ♀).	Anonymous 1 (1994a) (CA) B.6.1.1.1
Distribution and excretion EPA Guideline 85-1. Comparable to OECD TG 417 (2010) GLP: yes	Recovery of administered dose (AD): Recoveries ranged from 98 to over 100 %:	Anonymous 2 (1995a) (CA) B.6.1.1.2

Type of study/guideline, deviation if any/species/route/dosage/test substance	Results/Remarks	Reference
Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8% [5^{14}C] SL-160 (Pm) (pyrimidinyl-ring), Lot No. CP-1386, purity 97-98% **Species**: Rat, Sprague Dawley (♂,♀) **Vehicle**: Aqueous 0.75 % (w/v) methylcellulose **Route/Dose**: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (volume 10 ml/kg) **Observation period**: 7 days **Pilot study**: Not CO_2 or volatile organics were expired during pilot study. So expired air is not collected. **No. of animals**: 5 ♂ and 5 ♀ per dose level (2 rats/sex were used as control) **Radiactivity per animal**: 55 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times. **Study acceptable**	**Recovery of the Administered Dose** | Dose Level / Sex | Urine %AD | Cagewash %AD | Feces %AD | Tissues %AD | Total %AD | |---|---|---|---|---|---| | Low Male | 78.92 ±4.05 | 0.67 ±0.20 | 18.14 ±3.36 | 2.06 ±1.29 | 99.78 ±3.79 | | Low Female | 89.37 ±2.83 | 0.57 ±0.46 | 8.65 ±1.47 | 0.17 ±0.15 | 98.76 ±2.24 | | High Male | 77.64 ±1.75 | 0.55 ±0.21 | 23.93 ±1.54 | 0.76 ±0.60 | 102.89 ±0.97 | | High Female | 89.32 ±5.86 | 0.34 ±0.21 | 8.87 ±1.28 | 0.21 ±0.13 | 98.73 ±6.43 | Distribution Little radiolabel remained in the animals after 7 days. Similar pattern was observed in both dose level groups although overall females seem to have a different distribution than males, which suggests differences in metabolism. **Low dose**: Total radioactivity in tissues was lower in ♀ than ♂. The highest concentration of radioactivity in males was found in blood (0.52% AD), carcass (1.36 % AD) and muscle (0.42 % AD). In females, only 0.17 % AD was found in tissues distributed primarily in blood (0.03 % AD), carcass (0.12 % AD) and muscle (0.05 % AD). **High dose**: Total radioactivity in tissues was lower in ♀ than ♂. The highest concentration of radioactivity was found in blood in both sexes (0.19% AD in ♂ and 0.04 % AD in ♀), carcass (0.48 % AD in ♂ and 0.14 % AD in ♀) and muscle (0.15% AD in ♂ and 0.04 % AD in ♀) Excretion Most of the radiolabelled compound was excreted at 72 h post-dosing. The primary route was *via* urine and a minor route through faeces. The total excretion was almost completed 7 days after the administration. Similar pattern was observed in both dose level groups although significant differences between sexes, which suggests females have rapid excretion. **Low dose**: Urine was the main route of excretion in both sexes although at different percentages (79% AD in ♂ and 89 % AD in ♀). Excretion in faeces accounted for 18 % AD in ♂ and 9 % AD in ♀). **High dose**: Urine was the main route of excretion in both sexes although at different percentages (78% AD in ♂ and 89 % AD in ♀). Excretion in faeces accounted for 24 % AD in ♂ and 9 % AD in ♀)	
Pharmacokinetics EPA Guideline 85-1 Comparable to OECD TG 417 (2010) GLP: Yes Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8% [^{14}C]- SL-160 (P) (pyridinyl ring), Lot No. CP-1385, purity 97.29% **Species**: Rat, Sprague Dawley (SPF) (♂,♀)	Absorption: Rapid at low dose, fairly rapid at high dose Peak time (Tmax): Significantly different between dose levels. **Low dose**: 0.5 h ♂/♀. **High dose**: 6h ♂, 2-6 h ♀ Peak concentration (Cmax): Significantly different between dose levels, but not between sexes. **Low dose**: 6.76-8.27 µg-eq/g.h ♂, 7.04-8.61 µg-eq/g.h ♀. **High dose**: 141.98-164.09 µg-eq/g.h ♂, 126.41-156-94 µg-eq/g.h ♀). Elimination half-life ($T_{1/2}$): Significant differences between sexes and dose levels. Elimination at high dose was significantly longer than the low dose level. **Low dose**: 27.2 h ♂, 18.8 h ♀. **High dose**: biphasic curve,	Anonymous 3 (1995b) (CA) B.6.1.1.3

Type of study/guideline, deviation if any/species/route/dosage/test substance	Results/Remarks	Reference
Vehicle: aqueous 0.75% (w/v) methylcellulose **Route/Dose**: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (10 ml/kg volume) **Observation period**: 7 days **No. of animals**: 5 ♂ and 5 ♀ per dose level (1 rat/sex were used as control) **Radiactivity per animal**: 100 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times **Study acceptable**	α phase (distribution and elimination) 9.2 h ♂ and 8.0 h ♀; β phase (terminal elimination) 36 h ♂ and 33.8 h ♀. AUC: Significantly different between sexes and between dose levels. **Low dose**: 304 µg-eq/g.h ♂ and 189 µg-eq/g.h ♀. **High dose**: 4400 ♂ µg-eq/g.h and 3080 µg-eq/g.h ♀.	
Pharmacokinetics EPA Guideline 85-1 Comparable to OECD TG 417 (2010) GLP: Yes Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8% [5^{14}C] SL-160 (Pm) (pyrimidinyl-ring), Lot No. CP-1386, purity 97.29% **Species**: Rat, Sprague Dawley (♂,♀) **Vehicle**: aqueous (0.75% (w/v) methylcellulose **Route/Dose**: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (10 ml/kg volume) **Observation period**: 7 days **No. of animals**: 5 ♂ and 5 ♀ per dose level (1 rat/sex were used as control) **Radiactivity per animal**: 100 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times **Study acceptable**	Absorption: Fairly rapid at both dose levels Peak time (Tmax): Significantly different between dose levels. **Low dose**: 6 h ♂/♀. **High dose**: 4h ♂/♀ Peak concentration (Cmax): Significantly different between dose levels, but not between sexes. **Low dose**: 8-9 µg-eq/g.h ♂, 9-13 µg-eq/g.h ♀. **High dose**: 137-162 µg-eq/g.h ♂, 153-190 µg-eq/g.h ♀) Elimination half-life ($T_{1/2}$): Similar patterns observed in both sexes and at both dose levels. **Low dose**: 28 h ♂, 17 h ♀. **High dose**: 26 h ♂ and 17 h ♀. Combined (low+high): 26 h ♂, 17 h ♀ AUC: Significantly different between sexes. Males appear to have slower elimination rates since peak time and peak concentration were not significantly different between sexes. **Low dose**: 361 µg-eq/g.h ♂ and 259 µg-eq/g.h ♀. **High dose**: 6630 ♂ µg-eq/g.h and 5710 µg-eq/g.h ♀	Anonymous 4 (1995c) (CA) B.6.1.1.4
Biliary excretion EPA Guideline 85-1 Comparable to OECD TG 417 (2010) GLP: Yes Flazasulfuron (SL-160), Lot No.: Y-920205, purity 99.8% [^{14}C]- SL-160 (P) (pyridinyl ring), Lot No.: CP-1385, purity > 97% **Species**: Rat, Sprague Dawley (♂,♀) **Vehicle**: Aqueous (0.75% (w/v) methylcellulose	Material balance Recovery was good: 100 % AD (n=16). Biliary excretion (48 h): No significant difference between sexes or dose level groups. **Low dose**: 9.89 % AD ♂, 9.17 % AD ♀. **High dose**: 16.66 % AD ♂, 9.82 % AD ♀. Urinary excretion (48 h): Urine was the primary route of excretion. No significant difference between sexes at low dose group. However, at high dose group females excreted significantly more than males. **Low dose**: 31.38 % AD ♂, 42.98 % AD ♀. **High dose**: 30.02 % AD ♂, 63.02 % AD ♀. Faecal excretion (48 h):	Anonymous 5 (1995d) (CA) B.6.1.1.7

Type of study/guideline, deviation if any/species/route/dosage/test substance	Results/Remarks	Reference
Route/Dose: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (10 ml/kg volume) **Observation period**: 48 h **No. of animals**: 4 ♂ and 4 ♀ per dose level (1 rat/sex were used as control) **Radiactivity per animal**: 55 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times. **Study acceptable**	Minor route of excretion. No significant difference between sexes or dose levels. Total amount of radiolabel found in faeces was only 2.57-3.47 % AD ♂ and 3.04-3.26 % AD ♀. Radiolabel in tissues (48 h): Differences in residues between males and females, *i.e.* males contained more radiolabelled in carcass and blood. **Low dose**: Carcass (36.31 % AD ♂, 25.85 % AD ♀), blood (12.51 % AD ♂, 10.92 % AD ♀) **High dose**: Carcass (28.21 % AD ♂, 11.70 % AD ♀), blood (8.84 % AD ♂, 4.63 % AD ♀).	
Biliary excretion EPA Guideline 85-1 Comparable to OECD TG 417 (2010) GLP: Yes Flazasulfuron (SL-160), Lot No.: Y-920205, purity 99.8% [5^{14}C] SL-160 (Pm) (pyrimidinyl-ring), Lot. No.: CP-1386, purity >97% **Species**: Rat, Sprague Dawley (♂,♀) **Vehicle**: aqueous 0.75% (w/v) methylcellulose **Route/Dose**: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (10 ml/kg volume) **Observation period**: 48 h **No. of animals**: 4 ♂ and 4 ♀ per dose level (1 rat/sex were used as control) **Radiactivity per animal**: 55 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times **Study acceptable**	Material balance Recovery was good: 102 % AD (n=15). Biliary excretion (48 h): Males appear to excrete more radiolabel than females. **Low dose**: 16.99 % AD ♂, 8.43 % AD ♀. **High dose**: 13.53 % AD ♂, 10.93 % AD ♀. Urinary excretion (48 h): Urine was the primary route of excretion. No significant difference between sexes at a given dose level or between te dose group. **Low dose**: 34.62 % AD ♂, 42.25 % AD ♀. **High dose**: 37.51 % AD ♂, 52.37 % AD ♀. Faecal excretion (48 h): Minor route of excretion. No significant difference between sexes or dose levels. Total amount of radiolabel found in faeces was only 2.62-4.35 % AD ♂ and 2.66-3.19 % AD ♀. Radiolabel in tissues (48 h): Similar amounts between males and femalese at a given dose level. **Low dose**: Carcass (32.82 % AD ♂, 28.27 % AD ♀), blood (10.43 % AD ♂, 11.46 % AD ♀) **High dose**: Carcass (23.00 % AD ♂, 18.34 % AD ♀), blood (7.03 % AD ♂, 6.23 % AD ♀).	Anonymous 6 (1995e) (CA) B.6.1.1.8
Pilot Study – Excretion EPA Guideline 85-1 Comparable to OECD TG 417 (2010) GLP: Yes Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8 % [^{14}C]- SL-160 (P) (pyridinyl ring), Lot No.: CP-1385, Purity 97.8 % (P-label) [5^{14}C] SL-160 (Pm) (pyrimidinyl-ring), Lot No.: CP-1386, purity 97.2 % (Pm-label) **Species**: Rat, Sprague Dawley (♂,♀) **Reference substances**: ADMP, DTPP, DTPU, HTPP, TPSA	Material balance Recovery P-label groups: 90.2 to 100.9 % AD. Recovery Pm-label groups: 94 % to 104.7 % AD. Expired air excretion (48h): Less than 0.08 % AD for either pyridinyl- and pyrimidinyl-treatment groups. Collection of the expired air is not necessary. Urine excretion (48 h): Major route of excretion. No significant differences between P-label or Pm-label for a given sex. P-Label - **Low dose**: 55.34 % AD ♂, 63.97 % AD ♀; **High dose**: 56.19 % AD ♂, 72.93 % AD ♀ Pm-Label – Low dose: 44.27 % AD ♂, 69.17 % AD ♀; **High dose**: 54.37 % AD ♂, 73.05 % AD ♀ Faeces excretion (48 h): Minor route of excretion. No significant differences between P-label or Pm-label for a given sex.	Anonymous 7 (1996) (CA) B.6.1.1.9

<table>
<tr><th>Type of study/guideline, deviation if any/species/route/dosage/test substance</th><th>Results/Remarks</th><th>Reference</th></tr>
<tr><td><u>Vehicle</u>: aqueous (0,75% (w/v) methylcellulose
<u>Route/Dose</u>: Single oral dose (gavage), 2 mg/kg bw and 50 mg/kg bw (10 ml/kg volume)
<u>Observation period</u>: 48 h
<u>No. of animals</u>: 3 ♂ and 3 ♀ per dose level and compound (pyridinyl or pyrimidinyl), and 2 rats/sex as control
<u>Radiactivity per animal</u>: 50 to 65 µCi
<u>Animal handling</u>: Guide for the care and use of Laboratory animals is followed at all times.

Supporting information</td><td>P-Label - Low dose: 14.97 % AD ♂, 7.38 % AD ♀; High dose: 16.74 % AD ♂, 8.38 % AD ♀
Pm-Label – Low dose: 12.35 % AD ♂, 9.71 % AD ♀; High dose: 14.07 % AD ♂, 8.44 % AD ♀

<u>Radiolabel in tissues (48h)</u>:
Not significantly different between P-label and Pm-label groups for a given dose level and sex.
Total radioactivity in tissues:
P-Label - Low dose: 25.55 % AD ♂, 18.36 % AD ♀; High dose: 14.97 % AD ♂, 5.74 % AD ♀
Pm-Label – Low dose: 30.02 % AD ♂, 17.18 % AD ♀; High dose: 25.17 % AD ♂, 8.48 % AD ♀

Carcass, blood, GI tract, liver and kidneys contained high levels of radiolabel.
The percentage AD that remained in the blood was significantly different between P-label and Pm-label groups for both sexes at the low dose level:
P-Label - Low dose: 6.24 % AD ♂, 4.69 % AD ♀; High dose: 3.74 % AD ♂, 1.27 % AD ♀
Pm-Label – Low dose: 2.46 % AD ♂, 8.94 % AD ♀; High dose: 5.38 % AD ♂, 1.97 % AD ♀
The differences in blood radiolabel suggests the metabolism may differ depending on label compound and sex.

<u>Metabolite screening (urine)</u>:
Differences between P- and Pm-label metabolites.
Major metabolite ID as parent compound (SL-160)
Minor metabololiltes ID as TPSA, HTPP and DTPU. Additional non-identified metabolites may represent conjugates of SL-160
Differences in P- and Pm-label metabolites and also females appear to excrete more metabolites than males.

<u>Metabolite screening (faeces)</u>:
SL-160 was the major compound detected in faeces.
Major metabolite ID as HTPP. Additional metabolites detected but not characterised.</td><td></td></tr>
<tr><td>Metabolite ID

EPA Guideline 85-1. Comparable to OECD TG 417 (2010)

GLP Yes*
*NMR experiments. Some instrument data printouts were not signed and dated within one working day after the data generated. This deviation did not affect the integrity of the data and the results and conclusions of the study.

Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8%
[^{14}C]- SL-160 (P) (pyridinyl ring), purity 99.2 % (Batch No not reported)
[5^{14}C] SL-160 (Pm) (pyrimidinyl-ring), purity 98.9 %

<u>Species</u>: Rat, Sprague Dawley (♂,♀)
<u>Reference substances</u>:</td><td><u>Distribution of radioactivity (% AD) in 0-48 h urine and faeces</u>:
Very consistent across P- and Pm-labels.
Excretion of the majority of radioactivity was in urine (40 to 80 % AD) and faeces (7 to 20 % AD). Major urinary faeces and bile metabolites identified. No significant differences in radioactivity distribution between single dose and multiple dose rats.

<table>
<tr><th></th><th colspan="6">Pm Label</th></tr>
<tr><th></th><th colspan="3">Total 14C</th><th colspan="3">Total Metabolites</th></tr>
<tr><th></th><th>Urine</th><th>Feces</th><th>Total</th><th>Urine</th><th>Feces</th><th>Total</th></tr>
<tr><td>Single Dose</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Low Male</td><td>54.73</td><td>11.19</td><td>65.92</td><td>54.24</td><td>9.22</td><td>63.46</td></tr>
<tr><td>Low Female</td><td>77.56</td><td>7.18</td><td>84.74</td><td>77.53</td><td>5.66</td><td>83.18</td></tr>
<tr><td>High Male</td><td>66.37</td><td>20.16</td><td>86.53</td><td>58.49</td><td>17.32</td><td>75.81</td></tr>
<tr><td>High Female</td><td>81.76</td><td>7.94</td><td>89.69</td><td>81.58</td><td>6.89</td><td>88.47</td></tr>
<tr><td>Multiple Dose</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Low Male</td><td>52.35</td><td>15.66</td><td>68.02</td><td>52.14</td><td>15.37</td><td>67.51</td></tr>
<tr><td>Low Female</td><td>83.06</td><td>9.28</td><td>92.33</td><td>83.05</td><td>9.03</td><td>92.08</td></tr>
</table></td><td>Anonymous 8 (1995) (CA)
B.6.1.1.10</td></tr>
</table>

<table>
<tr><th>Type of study/guideline, deviation if any/species/route/dosage/test substance</th><th>Results/Remarks</th><th>Reference</th></tr>
<tr><td>ADMP, DTPP, DTPU, HTPP, TPSA, TPPG, MTMP, HDTP, HDPU and HDU.
<u>Vehicle</u>: aqueous 0.75% (w/v) methylcellulose
<u>Urine, faeces and bile samples</u> were collected at 2, 4, 6, 12, 24 and 48 h from the following studies:
-Single oral dose SL-160(P) and SL-160 (Pm) (B.6.1.1.1 and B.6.1.1.2)
-Repeat oral dose SL-160 (P) and SL-160 (Pm) (B.6.1.1.5 and B.6.1.1.6)
-Single oral dose (biliary extraction) SL-160 (P) and SL-160 (PM) (B.6.1.1.7 and B.6.1.1.8)

<u>Metabolite ID</u>:
SL-160, DTPU, HTPP and TPSA
Isolated from single high dose male P-label (urine)
HDPU
Isolated from single high dose male P-label (urine)
TPPG, HDTG and HDU
Isolated from single high dose male Pm-label (bile)
MTMG
Isolated from single high dose male P-label (urine)

<u>Dose</u>:
Low dose: 2 mg/kg bw
High dose: 50 mg/kg bw
<u>Animal handling</u>: Guide for the care and use of Laboratory animals is followed at all times.

Study acceptable</td><td>
<table>
<tr><th></th><th colspan="6">P Label</th></tr>
<tr><th></th><th colspan="3">Total 14C</th><th colspan="3">Total Metabolites</th></tr>
<tr><th></th><th>Urine</th><th>Feces</th><th>Total</th><th>Urine</th><th>Feces</th><th>Tol</th></tr>
<tr><td>Single Dose</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Low Male</td><td>47.38</td><td>12.15</td><td>59.53</td><td>47.37</td><td>11.43</td><td>58.</td></tr>
<tr><td>Low Female</td><td>73.76</td><td>7.30</td><td>81.06</td><td>73.76</td><td>6.72</td><td>80.</td></tr>
<tr><td>High Male</td><td>67.81</td><td>20.33</td><td>88.14</td><td>67.81</td><td>19.44</td><td>87.</td></tr>
<tr><td>High Female</td><td>87.60</td><td>8.47</td><td>96.07</td><td>87.60</td><td>7.91</td><td>95.</td></tr>
<tr><td>Multiple Dose</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Low Male</td><td>48.25</td><td>15.06</td><td>63.31</td><td>48.25</td><td>14.72</td><td>62.</td></tr>
<tr><td>Low Female</td><td>74.53</td><td>7.75</td><td>82.28</td><td>74.52</td><td>7.67</td><td>82.</td></tr>
</table>
<u>Urine metabolites (P-label)</u>:
SL-160 (parent) was the major compound (19 to 57 % AD). DTPU, HDPU, HTPP, TPSA, MTMG, HDTG and TPPG were identified.
<u>Urine metabolites (Pm-label)</u>:
SL-160 (parent) was the major compound (20 to 66 % AD). DTPU, HDPU, HTPP, ADMP, HDTG; TPPG and HDU were identified.
<u>Faeces metabolites (P-label)</u>:
SL-160 (parent) was the major compound (2 to 5 % AD). DTPU, HDPU, HTPP, TPSA, MTMG, HDTG and TPPG were identified.
<u>Faeces metabolites (Pm-label)</u>:
SL-160 (parent) was the major compound (1.20 to 3.35 % AD). DTPU, HDPU, HTPP, ADMP, HDTG; TPPG and HDU were identified.
HDU was not identified in the high dose group from the single oral dose study (♂,♀).
DTPU was not identified in repeat dose studies (♂,♀).

<u>Bile metabolites (P-label)</u>:
Samples contained a small percentage of radioactivity (< 17 % AD). TPPG and HDTG are the major metabolites in bile. Minor metabolites include parent (SL-160), HTPP, TPSA and MTMG.

<u>Metabolic pathways</u>:
Major metabolic reactions include:
-Intramolecular rearrangement
-Cleavage at sulphonylurea bridges
-Oxidation at the 5-position of the pyrimidine ring
-Displacement by glutathione and subsequent metabolic reactions on the glutathione group
-Conjugation with glucuronic acid</td><td></td></tr>
<tr><td colspan="3">IN VIVO REPEAT-DOSE</td></tr>
<tr><td>Distribution and excretion

EPA Guideline 85-1
Comparable to OECD TG 417 (2010)

GLP: Yes

Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8%
[^{14}C]- SL-160 (P) (pyridinyl ring), Lot No. CP-1385, purity 98.49%</td><td><u>Recovery of administered dose (AD)</u>:
The excretion of radiolabel was rapid and extensive for both sexes. Almost all the labelled was recovered at the end of the study.</td><td>Anonymous 9 (1994b)
(CA)
B.6.1.1.5</td></tr>
</table>

<table>
<tr><th>Type of study/guideline, deviation if any/species/route/dosage/test substance</th><th>Results/Remarks</th><th>Reference</th></tr>
<tr><td>Species: Rat, Sprague Dawley (♂,♀)
Vehicle: aqueous 0.75 % (w/v) methylcellulose
Route/Dose: Repeated oral dose (gavage): 2 mg/kg bw (10 ml/kg) unlabelled compound for 14 days followed by a single oral dose of 2 mg/kg bw (10 ml/kg) labelled compound.
Observation period: 7 days after radiolabelled dose
Pilot study: Not CO_2 or volatile organics were expired during pilot study. So expired air is not collected.
No.of animals: 5 ♂ and 5 ♀ (1 rat/sex were used as control)
Radiactivity per animal: 55 µCi per kg of bw
Animal handling: Guide for the care and use of Laboratory animals is followed at all times.

Study acceptable</td><td>Recovery of Administered Radiolabeled Dose
Mean ± Standard Deviation, n=5
<table><tr><th>Sex</th><th>Urine %AD</th><th>Cage Wash %AD</th><th>Feces %AD</th><th>Tissues %AD</th><th>Total %AD</th></tr><tr><td>Male</td><td>73.58 ±3.96</td><td>0.85 ±0.34</td><td>22.78 ±2.26</td><td>2.85 ±1.40</td><td>100.06 ±1.87</td></tr><tr><td>Female</td><td>90.02 ±5.55</td><td>1.41 ±1.17</td><td>9.75 ±1.76</td><td>0.52 ±0.13</td><td>101.70 ±4.69</td></tr></table>
Distribution:
Little radiolabel remained in the animals at the end of the study.
Similar distribution in tissues for both sexes although concentration was less in females: carcass (1.70 % ♂, 0.30 % ♀), blood (0.74 % ♂, 0.16 % ♀), liver (0.23 % ♂, 0.04 % ♀) and kidney (0.13 % ♂, 0.02 % ♀).

Excretion:
Overall elimination of the administered dose was more rapid in females than males.
Urine was the main route of excretion, which was more rapid and extensive in females than males: 74 % AD ♂, 90 % AD ♀.
Faeces was a minor route of elimination. Approximately 23 % AD ♂, 10 % AD ♀.
Differences in elimination rate between males and females suggest the metabolism is different across sex.

Comparison with single dose study (low dose) (B.6.1.1.1)
No significant differences ($p > 0.05$) between results from single and repeat-dose studies. The results demonstrated that the metabolism of orally administered flazasulfuron in rats was different between sexes and the elimination of the radiolabelled compound in rat was rapid and complete.</td><td></td></tr>
<tr><td>Distribution and excretion

EPA Guideline 85-1
Comparable to OECD TG 417 (2010)

GLP: Yes

Flazasulfuron (SL-160), Lot No. Y-920205, purity 99.8%
Labelled: [5^{14}C] SL-160 (Pm) (pyrimidinyl-ring), Lot No. CP-1386, purity >97.5%

Species: Rat, Sprague Dawley (♂,♀)
Vehicle: aqueous 0.75 % (w/v) methylcellulose
Route/Dose: Repeated oral dose (gavage): 2 mg/kg bw (10 ml/kg) unlabelled compound for 14 days followed by a single oral dose of 2 mg/kg bw (10 ml/kg) labelled compound.
Observation period: 7 days after radiolabelled dose
Pilot study: Not CO_2 or volatile organics were expired during pilot study. So expired air is not collected.
No. of animals: 5 ♂ and 5 ♀ (1 rat/sex were used as control)</td><td>Recovery of administered dose (AD):
The excretion of radiolabel was rapid and extensive for both sexes. Almost all the labelled was recovered at the end of the study.

Recovery of Administered Radiolabeled Dose
Mean ± Standard Deviation, n=5
<table><tr><th>Sex</th><th>Urine %AD</th><th>Cage Wash %AD</th><th>Feces %AD</th><th>Tissues %AD</th><th>Total %AD</th></tr><tr><td>Male</td><td>73.23 ±3.07</td><td>1.13 ±0.42</td><td>22.93 ±2.83</td><td>1.61 ±0.76</td><td>98.91 ±1.55</td></tr><tr><td>Female</td><td>91.17 ±1.51</td><td>0.55 ±0.37</td><td>9.01 ±1.38</td><td>0.29 ±0.07</td><td>101.01 ±1.32</td></tr></table>
Distribution:
Little radiolabel remained in the animals at the end of the study. The average amount of radioactivity remaining in tissues was 1.61 % AD ♂ and 0.29 % AD ♀.
Similar distribution in tissues for both sexes although concentration was less in females: carcass (1.08 % ♂, 0.23 % ♀), blood (0.35 % ♂, 0.02 % ♀), liver (0.14 % ♂, 0.02 % ♀) and kidney (0.02 % ♂, <0.005 % ♀).

Excretion:
Overall elimination of the administered dose was more rapid in females than males.
Urine was the main route of excretion, which was more</td><td>Anonymous 10 (1995f)
(CA)
B.6.1.1.6</td></tr>
</table>

Type of study/guideline, deviation if any/species/route/dosage/test substance	Results/Remarks	Reference
Radiactivity per animal: 55 µCi per kg of bw **Animal handling**: Guide for the care and use of Laboratory animals is followed at all times. **Study acceptable**	rapid and extensive in females than males: 73 % AD ♂, 91 % AD ♀. Faeces was a minor route of elimination. Approximately 23 % AD ♂, 9 % AD ♀. Differences in elimination rate between males and females suggest the metabolism is different across sex. Comparison with single dose study (low dose) (B.6.1.1.2) No significant differences ($p > 0.05$) between results from single and repeat-dose studies. The results demonstrated that the metabolism of orally administered flazasulfuron in rats was different between sexes and the elimination of the radiolabelled compound in rat was rapid and complete.	
IN VITRO		
Comparative *in vitro* metabolism There are no specific testing regulations / guidelines GLP: No Flazasulfuron (SL-160), Batch No. Y-920205, purity 100.0% **Solvent**: water **Liver microsomes**: Rat (Sprague-Dawley). Dog (Beagle) and Human **Dosage**: 1 and 50 µM **Incubation times**: 0, 10, 30, 60 and 120 min **Searched metabolites**: Demethylation Hydroxylation Demethylation + hydroxylation Dyhydroxylation GTPS,DMPU,HTPP,DTPP,DTPU,TPSA, HTPU,HTF,HMTU, SL-160 4,6-Dimethoxypyrimidin-2-amine 1-((3-(trifluoromethyl)pyridin-2-yl)methyl)guanidine **Study acceptable**	From the data generated in this study, there was no marked decrease in the amount of SL-160 following incubation with pooled rat, dog and human liver microsomes (< 10 % decrease after 120 minutes incubation compared to the decrease observed in the absence of microsomes), suggesting that SL-160 was relatively stable under the incubation conditions used. Up to nine (most minor, < 5 % of the total peak area) metabolites were detected in the incubation samples in addition to parent compound SL-160. The metabolites **SSRE-008 (HTPP), SSRE-004 (DTPU) and hydroxylated SL-160 (HDPU)** were detected in all species. **SSRE-006 (HMTU), SSRE-003 (DMPU)** and **SSRE-020 (HTPU)** were minor metabolites (< 5 % total peak area) observed at 50 µM only in human. **Hydroxylate SSRE-005 (HDTPP)** was not detected in human. **SSRE-005 (DTPP)** was not detected in dogs. **Hydroxylated SSRE-004** and **hydroxylated SSRE-005** were detected in rat and dog but not human. **GTPS, HTF** and **TPSA** were not found. (unclear metabolic pathway)	Anonymous 56 (2014) (CA) B.6.1.1.11

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

A series of studies have been conducted with radiolabelled flazasulfuron [^{14}C-SL-160 (P) and ^{14}C-SL-160(Pm)] at two dose levels (2 and 50 mg/kg bw) to evaluate the ADME properties of flazasulfuron in rats.

^{14}C-SL-160 (P)

Flazasulfuron-labelled pyridine ring, SL-160 (P), is absorbed fairly rapid, extensively distributed and excreted mostly in urine and faeces.

In a single oral dose study (Anonymous 1, 1994a; B.6.1.1.1) most of radiolabelled compound was excreted at 48 h except for the low dose male group. Urine was the major route of excretion ranging from 75-80 % AD in male rats and 94 % AD in female rats. Excretion in faeces accounted for 21 % AD in males and 10 % AD in

females. After 7 days post-labelling, very low radioactivity was recovered in tissues with the observation than females retained less amounts than males. Blood, muscle and carcass were found to retain most radiolabelled.

In a repeat dose study (Anonymous 9, 1994b; B.6.1.1.5) the excretion of radiolabel was rapid and extensive for both sexes. Most radiolabel recovered at the end of the study (7 days after radiolabel dose). The major route of excretion was urine, which was more rapid in females than males (74 % AD in males and 90 % in females). Faeces was the minor route of excretion and it was higher in males than females (23 % AD in males and 10 % AD in females). Distribution in tissues (the majority in carcass, blood, liver and tissue) was similar in both sexes although females retained less amounts.

Billiary excretion was investigated (Anonymous 5, 1995d; B.6.1.1.7) and the results indicated that there are no significant differences between sexes and dose levels (14-17 % AD in males and 8-11 % AD in females).

In a pharmacokinetic study (Anonymous 3, 1995b; B.6.1.1.3), ^{14}C-SL-160 (P) was absorbed fairly rapidly, T_{max} ranged from 30 min at low dose to 6h at high dose for both male and females. Elimination was also rapid although significant differences between sexes were observed (27 h for males and 19 h for females in the low dose group, 36 h for males, 33 h for females in the high dose group). The AUC was higher for males than females at both dose levels.

^{14}C-SL-160 (Pm)

Flazasulfuron-labelled pyridine ring, SL-160 (Pm), is absorbed fairly rapid, extensively distributed and excreted mostly in urine and faeces.

In a single oral dose study (Anonymous 2, 1995a; B.6.1.1.2) the majority of radiolabelled compound was eliminated within 72 h. Urine was the major route of excretion ranging from 78-79 % AD in males and 89 % in females. Faeces was the minor route of excretion and it was higher in males than females (18-24 % AD in males and 9 % AD in females). After 7 days post-labelling, very low radioactivity was recovered in tissues and similar distribution pattern across carcass, blood and muscle were found at both dose levels, although females retained less amounts than males.

In a repeat dose study (Anonymous 10, 1995f; B.6.1.1.6) the excretion of radiolabel was rapid and extensive for both sexes. Almost all the radiolabel was recovered at the end of the study. The major route of excretion was urine, which was more rapid in females than males (73 % AD in males and 91 % in females). Faeces was the minor route of excretion and it was higher in males than females (23 % AD in males and 9 % AD in females). Distribution in tissues (the majority in carcass, blood, liver and tissue) was similar in both sexes although females retained less amounts.

Billiary excretion was investigated (Anonymous 6, 1995e; B.6.1.1.8) and the results indicated that males excreted higher amounts in bile than females: 14-17 % AD in males and 8-10 % AD in females.

In a pharmacokinetic study (Anonymous 4, 1995c; B.6.1.1.4), ^{14}C-SL-160 (Pm) was absorbed fairly rapidly, T_{max} ranged from 6 h in the low dose group to 4 h in the high dose groups for both males and females. Elimination was also rapid although significant differences between sexes were observed and females eliminated faster than males (28 h for males and 17 h for females in the low dose group, 26 h for males, 17 h for females in the high dose group).The AUC was higher for males than females at both dose levels.

Metabolism of flazasulfuron

A pilot study was conducted (Anonymous 7, 1996; B.6.1.1.9) to investigate the elimination of radiolabel in expired air and also the potential differences in metabolism for the pyrimidine (P)- and pyrimidinyl (Pm)-labelled flazasulfuron (SL-160). The results indicated that less than 0.08 % AD for either P- and Pm-labelled treament groups, and therefore, no need to collect expired air was concluded. Urine was the major route of elimination and faeces was a minor route of elimination. In both urine and faeces, there were no significant differences between P- and Pm-labelled treatment groups for a given sex.

The metabolism *in vivo* of flazasulfuron (SL-160) was investigated (Anonymous 8, 1995; B.6.1.1.10). The parent compound (SL-160) was identified as the major radioactive component in urine and faeces.

The major metabolites in urine were the glucuronide derivatives TPPG and HDTG (see figure below). Additional metabolites were identified as DTPU, HTPP and TPSA and the minor metabolites included HDPU, MTMG, ADMP and HDU.

In faeces, the major metabolites were also the conjugates TPPG and HDTG. Bile samples contained TPPG, HDTG and a small amount of parent compound SL-160.

Flazasulfuron undergoes the following major metabolic reactions in rats:

- Intramolecular rearrangement
- Cleavage at sulphonylurea bridges
- Oxidation at the 5-position of the pyrimidine ring
- Displacement by glutathione and subsequent metabolic reactions on the glutathione group
- Conjugation with glucuronic acid

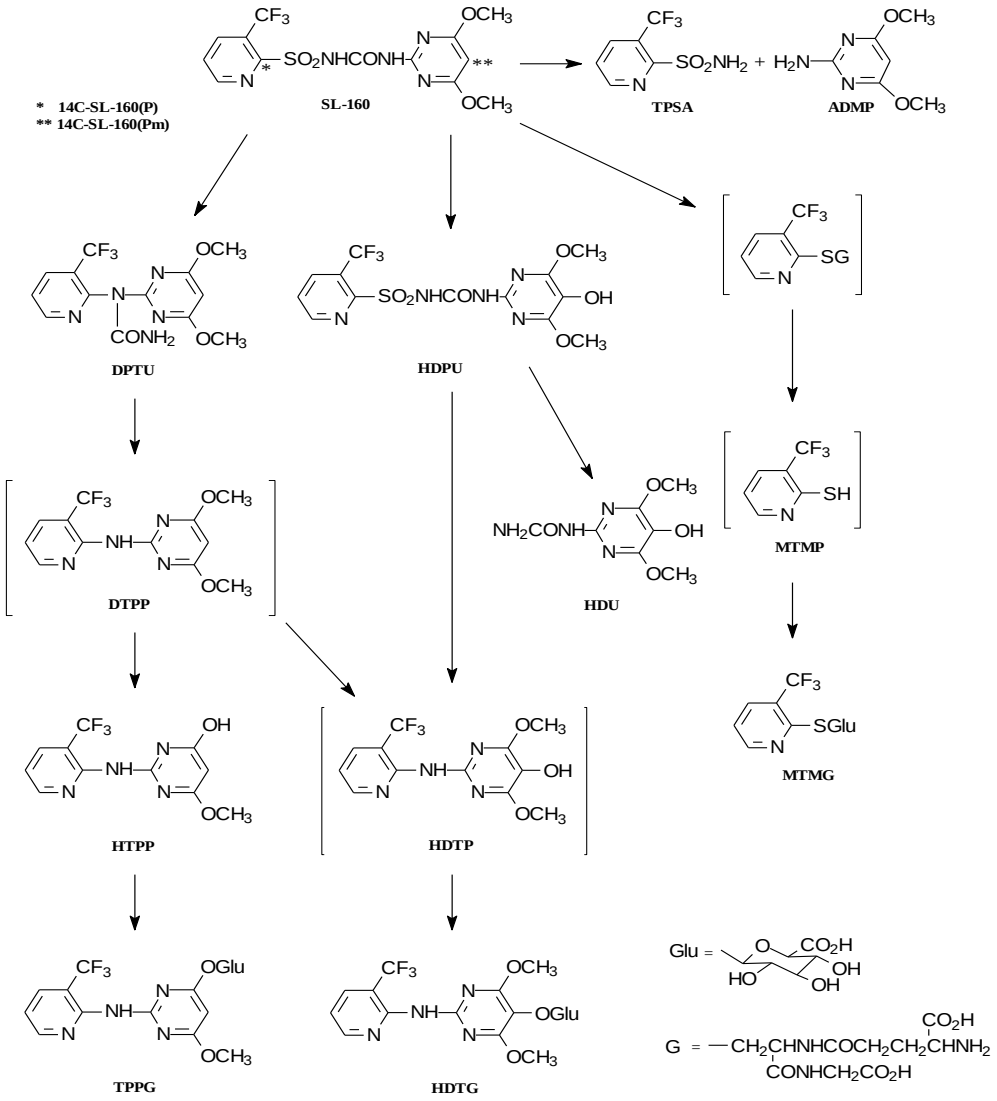
The full metabolic pathway in rats is shown in Scheme 1.

A comparative i*n vitro* metabolism study using rat, dog and human microsomes was conducted (Anonymous 56, 2014; B.6.1.1.11). Up to nine metabolites (most minor, *i.e.* < 5% total peak area) were detected in the incubation samples in addition to parent compound (SL-160).

The metabolites HTPP, DTPU and hydroxylated flazasulfuron (HDPU) were detected in all species. The outcome of the study indicated that three minor metabolites were detected in human microsomes at the 50 µM treatment (< 5 % total peak area): HMTU (SSRE-006) at 1.1 %, DMPU (SSRE-003) at 0.4 % and HTPU (SSRE-20) at 0.3 %.

Following the review of flazasulfuron at EU level (dRAR 2016) a data gap was identified for these three unique human metabolites for pesticides (EFSA Peer Review Report on Flazasulfuron, August 2016).

Scheme 1: Metabolic pathway of flazasulfuron in rats



10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 25: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Test substance, species, strain, sex, no/group, dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study in rats Method comparable to OECD TG 401 GLP: Yes **Study acceptable** Deviation: Fasting	Flazasulfuron (Purity: 96.3%) Rat Sprague Dawley 10/sex/group Doses: 2500 and 5000 mg/kg bw Fasting: over-night	> 5000 mg/kg bw (male and female) No mortality, clinical signs nor gross findings were observed. All animals exhibited bw gain after 7 and 14 days. **LD_{50}:** > **5000** mg/kg bw for both sexes	Anonymous 11 (1988a) (CA) B.6.2.1.1

Method, guideline, deviations if any	**Test substance, species, strain, sex, no/group, dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
until the end of administration to all animals (in Guidelines: 3-4 hours after administration)	Single dose (gavage) 14-day observation period		
Acute oral toxicity study in mice Method comparable to OECD TG 401 GLP: Yes **Study acceptable** Deviation: Fasting until the end of administration to all animals (in Guidelines: 3-4 hours after administration)	Flazasulfuron (Purity: 96.3%) Mouse ICR (Crj:CD-1) 10/sex/group Doses: 2500 and 5000 mg/kg bw Fasting: 2h Single dose (gavage) 14-day observation period	> 5000 mg/kg bw (male and female) No mortality, clinical signs nor gross findings were observed. All animals showed bw gain after 14 d compared with the bw before treatment. (After 7 d, 3♀ showed slight weight loss). **LD_{50}:** > **5000** mg/kg bw for both sexes	Anonymous 12 (1988b) (CA) B.6.2.1.2

Table 26: Summary table of human data on acute oral toxicity

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data available				

Table 27: Summary table of other studies relevant for acute oral toxicity

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data available				

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Two studies on acute oral toxicity with flazasulfuron are available: one in rats and one in mice. Both studies were performed in compliance with the OECD requirements for GLPs and Guidelines that were in force at the time.

The followed OECD TG 401 on acute oral toxicity was deleted in 2002 in order to introduce more refined methods reducing the number of test animals but the results are considered appropriate for the assessment of the acute oral toxicity of flazasulfuron. Both studies show similar results, with no deaths nor clinical signs of toxicity, and for both species the LD_{50} is greater than 5000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

Based on the available studies, the oral LD_{50} of flazasulfuron is greater than 5000 mg/kg bw in rats (Anonymous 11, 1988a; B.6.2.1.1) and mice (Anonymous 12, 1988b; B.6.2.1.2), which is above the threshold value of 2000 mg/kg bw for triggering acute oral toxicity classification according to CLP criteria.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Data available indicates that flazasulfuron does not meet the classification criteria for acute oral toxicity.

10.2 Acute toxicity - dermal route

Table 28: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Test substance, species, strain, sex, no/group, dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study in rats Method comparable to OECD TG 402 GLP: Yes **Study acceptable**	Flazasulfuron (Purity: 96.3%) Rat SD (Crj:CD) 10/sex/group Doses: 1000 and 2000 mg/kg bw Single dose 24-h exposure period 14-d observation period	> 2000 mg/kg bw (male and female) No mortality, clinical signs nor gross findings were observed. All animals exhibited bw gain after 7 and 14 days. Conclusion: **LD_{50} >2000** mg/kg bw for both sexes	Anonymous 13 (1988) (CA) B.6.2.2

Table 29: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 30: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

The available study on acute dermal toxicity with flazasulfuron (Anonymous 13, 1988; B.6.2.2) was performed in compliance with the OECD requirements for GLPs and Guidelines that were in force at the time. The main procedure is equivalent to the current testing guideline: solid test substance moistened with water, applied in approximately a 10% of the skin surface for a 24-hour exposure period, after which the remaining test substance was removed with water and a period of observation of 14 days. Under these conditions, no clinical signs of toxicity, mortality nor gross pathological lesions were found in any of the treated animals (both at 1000 and 2000 mg/kg bw dose groups), even bodyweight gain was observed in all the animals through the study (7 and 14 days after treatment).

RMS considers the result obtained in this study (dermal LD_{50} greater than 2000 mg/kg bw in rats) is suitable for the assessment of the acute dermal toxicity of flazasulfuron.

10.2.2 Comparison with the CLP criteria

Based on the available study (Anonymous 13, 1988; B.6.2.2), the dermal LD_{50} of flazasulfuron is greater than 2000 mg/kg bw in rats, which is above the threshold value of 2000 mg/kg bw for triggering acute dermal toxicity classification according to CLP criteria.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Data available indicates that flazasulfuron does not meet the classification criteria for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Table 31: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group, duration of exposure	Test substance, dose levels, form and particle size (MMAD)	Value LC_{50}	Reference
Acute inhalation toxicity study in rats Method comparable to OECD TG 403 GLP: Yes **Study acceptable** Deviations: No observations during exposure due to foggy dusts in the chamber.	Rat Fischer (F344/DuCrj) 10/sex/group Single whole-body exposure (dust) 4-hour exposure period 14-day observation period	Flazasulfuron (Purity: 96.4%) Atmosphere characteristics: (mean values): Concentration: 5.99 mg/L MMAD: 4.0 µm GSD: 2.1	> 5.99 mg/L/4 hour No mortality. No abnormalities observed at necropsy. Clinical signs: After exposure (males and females): Chromodacryorrhea and wetness around nose and mouth, on thoracic fur and around anus. These signs disappeared by post- exposure day 1. From 2 – 3 h after exposure (males and females): Reddish brown stains around the nose and mouth, that disappeared by day 6. From day 9 (all females) alopecia around the eyes, but recovered by day 14. All signs disappeared at the end of the observation period. Conclusion: **LC_{50} > 5.99** mg/L/4hour	Anonymous 14 (1988) (CA) B.6.2.3

Table 32: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 33: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The available study on acute inhalation toxicity with flazasulfuron (Anonymous 14, 1988; B.6.2.3) was performed in compliance with the OECD requirements for GLPs and Guidelines that were in force at the time.

The exposure conditions and data provided are considered adequate to evaluate and, therefore, the resulting LC_{50} of > 5.99 mg/L/4h is used in the assessment of the acute inhalation toxicity of flazasulfuron.

10.3.2 Comparison with the CLP criteria

Based on the available study (Anonymous 14, 1988; B.6.2.3), the inhalation LC_{50} of flazasulfuron is greater than 5.99 mg/L/4h in rats, which is above the threshold value (for dusts or mists) of 5 mg/L/4 h for triggering acute inhalation toxicity classification according to CLP criteria.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Data available indicates that flazasulfuron does not meet the classification criteria for acute inhalation toxicity.

10.4 Skin corrosion/irritation

Table 34: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Skin irritation study in rabbits US EPA 81-5 comparable to OECD TG 404 GLP: Yes **Study acceptable** Deviations: 6 animals instead of 3	Rabbit, New Zealand white, 3/sex 6 rabbits	Flazasulfuron (Purity: 97.3%)	0.5 g test substance (moistened with 0.5 ml of deionised water) 4 h exposure period Dermal observation: 30 min and 1, 24, 48 and 72 h after the patch removal	No erythema, oedema or other dermal effects were observed in any of the 6 rabbits Mean scores (24/48/72h) were 0.0 for both, erythema and oethema, in all 6 animals. Conclusion: **Not skin irritant**	Anonymous 15 (1993a) (CA) B.6.2.4

Table 35: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 36: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

One skin irritation/corrosion study was included for the assessment of flazasulfuron (RAR 2016). This study was performed according OECD TG 404, GLP and following the US EPA FIFRA 81-5 guideline.

No skin alteration was observed after the application of flazasulfuron in any of the 6 animals. The mean value for erythema and oedema (24, 48 and 72 hours) was 0.0 for the six animals.

10.4.2 Comparison with the CLP criteria

The available skin irritation/corrosion study with flazasulfuron showed no skin lesions, with mean scores of 0.0 for erythema and oedema in the six animals. No other skin alterations were observed during the study.

The major criterion, according to Regulation 1272/2008 for the skin irritation category is that at least 2 of 3 tested animals have a mean score of ≥ 2.3 and ≤ 4.0 for erythema&eschar or for oedema. Since no skin reaction was observed in the available study, none of the criteria established in Regulation 1272/2008 for classification as irritant to skin is fulfilled.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Data available indicates that flazasulfuron does not meet the classification criteria for skin corrosion/irritation.

10.5 Serious eye damage/eye irritation

Table 37: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance, species, strain, sex, no/group, dose levels, duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>Eye irritation study in rabbits
US EPA 81-4 comparable to OECD TG 405
GLP: Yes
Study acceptable
Deviations: 9 animals instead of 3</td><td>Flazasulfuron (Purity: 97.3%)
Rabbit,
New Zealand white,
-Unwashed group:3/sex - Washed group: 3f
Dose: 0.1 g (grounded to a fine powder)
-Washed group: 2.5 min. after instillation both eyes were flushed with 100 ml of deionised water.
- Unwashed group: eyes remainded unwashed.</td><td>Results of animals with unwashed eyes after instillation:
<table>
<tr><td rowspan="3">Obs. time</td><td colspan="4">Rabbit 1 (M)</td><td colspan="4">Rabbit 2 (M)</td><td colspan="4">Rabbit 3 (M)</td></tr>
<tr><td rowspan="2">Cornea</td><td rowspan="2">Iris</td><td colspan="2">Conj.</td><td rowspan="2">Cornea</td><td rowspan="2">Iris</td><td colspan="2">Conj.</td><td rowspan="2">Cornea</td><td rowspan="2">Iris</td><td colspan="2">Conj.</td></tr>
<tr><td>E*</td><td>O*</td><td>E*</td><td>O*</td><td>E*</td><td>O*</td></tr>
<tr><td>24 h</td><td>0</td><td>0</td><td>2</td><td>1</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>48 h</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>72 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean 24/48/72 h</td><td>0</td><td>0</td><td>1</td><td>0.33</td><td>0</td><td>0</td><td>0.67</td><td>0</td><td>0</td><td>0</td><td>0.67</td><td>0</td></tr>
<tr><td rowspan="3">Obs. time</td><td colspan="4">Rabbit 4 (F)</td><td colspan="4">Rabbit 5 (F)</td><td colspan="4">Rabbit 6 (F)</td></tr>
<tr><td rowspan="2">Cornea</td><td rowspan="2">Iris</td><td colspan="2">Conj.</td><td rowspan="2">Cornea</td><td rowspan="2">Iris</td><td colspan="2">Conj.</td><td rowspan="2">Cornea</td><td rowspan="2">Iris</td><td colspan="2">Conj.</td></tr>
<tr><td>E*</td><td>O*</td><td>E*</td><td>O*</td><td>E*</td><td>O*</td></tr>
<tr><td>24 h</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>48 h</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>72 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean 24/48/72 h</td><td>0</td><td>0</td><td>0.67</td><td>0</td><td>0</td><td>0</td><td>0.33</td><td>0</td><td>0</td><td>0</td><td>0.67</td><td>0</td></tr>
</table>
* E: erythema / O: oedema
Additional ocular finding: observed 24 h after instillation in Rabbit 1 (M), described as purulent discharge. This finding was not observed in any other animal or time-point.

The study was finalised 72 h after instillation, since no ocular effects were observed in any of the rabbits at this moment.

Conclusion: Not eye irritant</td><td>Anonymous 16
(1993b)
(CA)
B.6.2.5</td></tr>
</table>

Table 38: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 39: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

One eye irritation/corrosion study was included for the assessment of flazasulfuron (RAR 2016). This study was performed according to GLP and following the US EPA FIFRA 81-4 guideline.

No corneal or iridial effects were observed in any rabbit throughout the study. Conjunctival irritation was observed in the form of erythema in all rabbits (mean scores of 1 (one rabbit), 0.67 (four rabbits) or 0.33 (one rabbit)) and oedema in one rabbit (mean score of 0.33). Also, purulent discharge was observed in one rabbit 24 hours after instillation, this finding was not observed in any other rabbit or observation time-point.

10.5.2 Comparison with the CLP criteria

According to the CLP criteria when 6 rabbits are used in the eye irritation study the test material is considered irritant to the eye when conjunctival erythema or oedema is ≥ 2 in at least 4/6 animals. The erythema of 0.67 in 4/6 animals obtained in the study does not meet the criteria for classification as irritating to the eyes according to CLP.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Data available indicates that flazasulfuron does not meet the classification criteria for serious eye damage/irritation.

10.6 Respiratory sensitisation

Table 40: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No data available					

Table 41: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 42: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data available.

10.6.2 Comparison with the CLP criteria

No data available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Data lacking.

10.7 Skin sensitisation

Table 43: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	**Test substance, species, strain, sex, no/group**	**Dose levels, duration of exposure and results**	**Reference**
Skin sensitisation (Buehler test) in guinea pig US EPA 81-6 comparable to OECD TG 406 GLP: No **Study not acceptable since a draft** report was provided with no signatures.	Flazasulfuron (Purity: 97.5%) Guinea pig Hartley Treated group: 10/sex Control: 5/sex Positive: 5/sex/group (DNCB and negative)	Dose levels: 100% (Induction and challenge phases). 3 induction applications in 3 weeks (6 h exposure period): 0.4 ml (mixture of 0.4 g moistened with 0.4 ml deionised water) Results: Control group: 2/10 (20%) animals showed slight skin responses (graded as ±[1]) at 24 h. No responses were observed at 48 h. Treated group: 6/20 animals (30%) showed slight skin responses (graded as ±[1] and regarded as barely perceptible) at 24 h, which lasted until 48 h in 3/20 animals (15%). However, these weak responses graded as ± are not considered as positive skin sensitisation responses and it has to be noted that it also occured also in the negative control group (20%). Positive control: all 10 animals showed skin responses grade 1 or 2 at 24 h which lasted as grade 1 at 48 h (except for 1 animal, graded as ±[1]). [1] *This response (±) was reported in the original study as "very faint (barely perceptible) to faint erythema".* Conclusion: **Not sensitising**	Anonymous 17 (1995) (CA) B.6.2.6.1
Guinea pig maximisation test (GPMT) Guideline: US EPA 81-6 comparable to OECD TG 406 GLP: Yes **Study**	Flazasulfuron (Purity: 97.5%) Hartley Treated group: 20 females Control 20	Preliminary test: Intradermal injection (induction): 3 dosing injections of each [2] and [3] (see table below) were administerd to each skin site of 4 animals, at doses of 0.5, 1 and 2.5 %. Results (individual scores not reported): irritation (including moderate and diffuse redness or scattered mild redness) was observed on the skin sites in all 4 animals injected with 2.5% or lower dosing solutions of both injections [2] and [3]. Topical application (induction and challenge): 2 dosing mixtures of 5 and 50% were applied to the flanks of 4 animals, and another 2 dosing mixtures of 10 and 25% were applied to other 4 animals (48 hours	Anonymous 18 (1998) (CA) B.6.2.6.2

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance, species, strain, sex, no/group</th><th>Dose levels,
duration of exposure and results</th><th>Reference</th></tr>
<tr><td>acceptable</td><td>females
Positive:
10 fem/group (DNCB and negative)</td><td>exposure period). Skin reactions were observed 24 h after the patch removal. Results (individual scores not reported): No irritation was observed in any animal given 50% or lower mixtures.
<u>Main test:</u>
<table>
<tr><th>Induction intradermal injection Day 0</th><th>Test</th></tr>
<tr><td>[1] 0.1 ml</td><td>FCA (Freund’s Complete Adyuvant)</td></tr>
<tr><td>[2] 0.1 ml</td><td>2.5% Suspension in paraffin oil</td></tr>
<tr><td>[3] 0.1 ml</td><td>2.5% Suspension (in paraffin oil) mixed with FCA in proportion 1:1</td></tr>
<tr><td>*Induction topical application Day 7 (occlusive patch 48 h)</td><td>0.4 g of the test substance mixture (50 % in white petrolatum)</td></tr>
<tr><td>Challenge topical application Day 21 (occlusive patch 24 h)</td><td>Left flank: 0.2 g of the test substance mixture (50 % in white petrolatum)
Right flank: 0.2 g of white petrolatum</td></tr>
</table>
*Application site of treated and negative control groups were rubbed with 10% sodium lauryl sulfate with white petrolatum the previous day (Day 0)
Control group had the same treatment on day 1 and 7 using white petrolatum instead of active substance.
Concurrent positive control was conducted with DNCB, which confirmed the validity of the test system.
<u>Results</u>
No skin reactions in any treated animal at 24 or 48 h after the patch removal (0/20). No response was observed in the controls either (0/20).
Conclusion: Not sensitising</td><td></td></tr>
</table>

Table 44: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 45: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Two studies on skin sensitisation with flazasulfuron are available. One of the provided reports is a draft-report which is not signed and, therefore, it is not considered acceptable (Anonymous 17, 1995; B.6.2.6.1). The other

study is a GLP Maximisation test in guinea pig performed according to guidelines comparable to OECD TG 406 giving negative results (Anonymous 18, 1998; B.6.2.6.2).

Moreover, human medical sourveillance data does not describe any skin sensitisation case.

10.7.2 Comparison with the CLP criteria

Negative results were obtained in a skin sensitisation (Maximisation Test) study in guinea pig (Anonymous 18, 1998; B.6.2.6.2). Besides, human medical surveillance data does not describe any skin sensitisation case.

Based on the available information, flazasulfuron is not a skin sensitiser.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Data available indicates that flazasulfuron does not meet the classification criteria for skin sensitisation.

10.8 Germ cell mutagenicity

Table 46: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Bacterial gene mutation (Ames test) FIFRA US EPA (1983), OECD (1981), Ministry of Labour Japan (1985) Comparable to OECD TG 471 (1997) Deviations from current OECD TG 471 (2020): The titre, the number of expected revertants, the checking of the aminoacid requirements, the R-factor plasmids presence, and the characteristic mutations are not reported. GLP: Yes **Study acceptable**	*S. typhimurium* TA100, TA1535, TA98, TA 1537 *E. coli* WP2 *uvrA* S9 from livers of rats induced with Aroclor 1254 Plate incorporation	Flazasulfuron technical (SL-160) Purity: 96.3 % Solvent: Sterile pure water Doses *S. typhimurium*: 2, 5, 10, 20, 50, 100, 200 µg/plate Doses *E. coli* WP2 uvrA: 100, 200, 500, 1000, 2000, 5000 µg/plate	Negative	Cytotoxicity observed in all S.typhimurim at doses 100 µg/plate and over. Cytotoxicity observed in *E.Coli* at 5000 µg/plate. 2-AA was used as positive control in all strains with metabolic activation. According to the OECD TG 471 (2020) an additional positive control should have been used.	Anonymous 57 (1987) (CA) B 6.4.1.1

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Mammalian cell gene mutation assay (Mouse lymphoma assay) EPA Guidelines 84-1; 84-2 Comparable to OECD TG 490 (2016) <u>Deviations from current OECD TG 490 (2016)</u> : *Colonies* -No numerical data about the size of the colonies. *Controls* -Some mutant frequencies of the negative controls are under the acceptability criteria of the author -EMS was used as positive control in the non-activation study (-S9) instead of MMS GLP: Yes **Study acceptable**	L5178Y $TK^{+/-}$ mouse lymphoma cells, clone 3.7.2.C. S9 derived from livers of adult male Sprague-Dawley rats induced with a mixture of Aroclor 1242 and Aroclor 1254 Positive controls: EMS and 7,12 DMBA	Flazasulfuron technical (SL-160, Lot 303) Purity: 97.1-97.5 % Solvent: Acetone Preliminary toxicity test: 9 doses ranging from 0.1-1250 µg/mL Initial & Confirmatory experiment: 20, 30, 40, 50, 60, 70, 80, 90, 100, 500 µg/mL	Negative	Due to solubility limitations, a concentration of 5000 µg/mL could not be achieved (highest tested dose was 1250 µg/mL) in the preliminary toxicity test Precipitation occurred at doses greater than 100 µg/mL	Anonymous 59 (1993) (CA) B.6.4.1.2
***In vitro* Chromosome Aberration assay** Comparable to OECD TG 473 (1997) <u>Deviations from current OECD TG 473 (2016)</u>: *Chemical* -Osmolality neither pH or precipitation are measured. -Cytotoxicity is based on mitotic index. The mitotic index is only an indirect measure of cytotoxic/cytostatic	Chinese hamster lung (CHL) cells S9 from liver of male Sprague Dawley rats induced with Aroclor 1254 Positive controls: mitomycin C and benzo(a) pyrene	SL-160 Technical (Lot No. 8706) Purity: 96.3 % Solvent: Sterile water <u>Growth inhibition test</u> 1.0 x 10^{-2}, 3.3 x 10^{-3}, 1.0 x 10^{-3}, 3.3 x 10^{-4} and 1.0 x 10^{-4} M 48 h treatment –S9 1 x 10^{-2} M 6 h Treatment +S9, 18 h growing with fresh medium. <u>Cytogenetic tests</u> <u>Without S9: (M)</u> 3.3 x 10^{-4}, 1.7 x 10^{-4},	Negative	The top concentration tested in the absence of S9 produced 52.5% of cell viability. The top concentration tested in the presence of S9 produced 69.5% of viability There is no short time experiment without S9 (3/6 hours) reported	Anonymous 60 (1988) (CA) B.6.4.1.3

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
effects and depends on the time after treatment *Analysis* -Short-term test without metabolic activation (3/6 h) is not reported -The number of metaphases is 200 as per EPA and OECD TG 473 (1997) Guidelines. However, the current OECD TG 473 (2016) establishes 300 metaphases. GLP: Yes **Study acceptable as supporting information**		8.3×10^{-5}, 4.1×10^{-5} and 2.1×10^{-5} M (direct method, continuous defined treatment, 24 and 48 h harvest time) <u>With S9 (M)</u> 1.0×10^{-2}, 5.0×10^{-3}, 2.5×10^{-3}, 1.3×10^{-3} and 6.3×10^{-4} M (6 h treatment, 12 and 18 h harvest time)			
***In vitro* Chromosome Aberration assay** OECD TG 473 (1997) <u>Deviations from current OECD TG 473 (2016)</u>: *Chemical* The selected concentrations do not cover the appropiate range according to the OECD TG 473 (2016) *Analysis* Relative cell count is reported as opposed to RICC recommended by the OECD TG 473 (2016) The number of metaphases is 200 as opposed to 300, according to the OECD TG 473	Chinese hamster CHL/IU cells Rat liver S9 mix Positive controls: mitomycin C and benzo(a) pyrene	Flazasulfuron (SL-160 technical) Purity: 97.9 % (analysed under non GLP) Solvent: Physiological saline <u>Short term treatment (6-18 h) (± S9)</u>: 256, 513, 1025, 2050 and 4100 µg/mL <u>Continuous treatment, 24h (-S9):</u> 64.1, 128, 256, 513 and 1025 µg/mL	Negative	More than one concentration produces turbidity. Precipitation was observed at 513 µg/mL and higher concentrations. For the short-term assay the concentrations were increased and for the long-term they were decreased.	Anonymous 61 (2014) (CA) B.6.4.1.4

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
(2016) Structural aberrant metaphases are not separated in poliploids and endoreduplicants GLP: Yes **Study acceptable**					
Rec-assay EPA guideline. OPPTS 870.5500 Fit-for-purpose GLP: Yes **Study acceptable as additional information**	*B. subtilis*, the recombination-wild (H17 Rec+) and the recombination-deficient (M45 Rec) S9 derived from livers of male Sprague-Dawley rats induced with Aroclor 1254 Negative control: kanamycin Positive controls: Mitomycin C and 2-amino-anthracene (2-AA)	Flazasulfuron technical (SL-160) Purity: 96.3% Solvent: Sterile water Doses: 20,50, 100, 200, 500, 1000 µg/disk	Negative	The study should be Disc Diffussion DNA repair proficient/deficient bacteria OPPTS 870.5500. No laboratory's historical database or supporting experiment for justifying the top concentration selected to be tested.	Anonymous 58 (1987) (CA) B.6.4.1.5

Table 47: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
Mammalian chromosome aberration in somatic cells (Micronucleus test) FIFRA Guideline 84-2 Comparable to OECD TG 474 (1983) <u>Deviations from current OECD TG 474 (2016)</u>: The number of polychromatic erythrocytes per	ICR Mice (♀,♂)	Flazasulfuron, SL-160 (Lot 303) Purity: 97.3 % Vehicle: 0.5 % carboxymethylcellulose in sterile distilled/deionised water Single dose by oral gavage Preliminary dose-range study: 5000 mg/kg/bw (5♀,5♂) 1, 10, 100, 1000 mg/kg/bw (2♂) Main study:	Negative		Anonymous 19 (1995) (CA) B.6.4.2

Method, guideline, deviations if any	Test system	Test substance and dosage	Results	Remarks	Reference
animal is 1000 as opposed to 4000 recommended by guidance for the incidence of micronucleated immature erythrocytes. GLP: Yes **Study acceptable**		Single dose (volume 20 mL): 0, 1250, 2500, 5000 mg/kg bw (5♀,5♂) Bone marrow cells collected at 24, 48 and 72 h following dose administration			

Table 48: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The potential genotoxicity of flazasulfuron has been investigated in an appropriate battery of *in vitro* assays and in one *in vivo* genotoxicity assay. *In vitro* tests for gene mutation (Ames test, mouse lymphoma cell assay), chromosomal aberration (cytogenetic assay in CHL cells) and DNA repair (rec-assay) and an *in vivo* assay for chromosomal aberration (mouse micronucleus test) were conducted.

In vitro:

Flazasulfuron was negative in both bacterial and mammalian cell gene mutation assays. Flazasulfuron did not induce chromosomal aberrations *in vitro* in two cytogenetic assays using CHL cells or CHL/UI cells, respectively. Flazasulfuron was also negative in the DNA repair (rec-assay).

In vivo:

Flazasulfuron was negative in an *in vivo* micronucleous assay (Anonymous 19, 1995; B.6.4.2). In a preliminary study, male and female mice were dosed up to 5000 mg/kg/bw and no clinical signs were observed. Hence, the maximum dose level was set at 5000 mg/kg/bw. Bone marrow cells, collected at 24, 48 and 72 h after dosing were examined for micronucleated polychromatic erythrocytes. Under the conditions of this study, flazasulfuron SL-160 did not induce a significant increase in micronucleated polychromatic erythrocytes in either male or female mice, hence it is deemed negative in the mouse micronucleus assay.

10.8.2 Comparison with the CLP criteria

No human data are available for flazasulfuron, hence a classification as Category 1A is not possible.

The classification in Category 1B is based on:
- positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or
- positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by

demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.

The classification in Category 2 is based on:
- positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:
- somatic cell mutagenicity tests *in vivo*, in mammals; or
- other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

All available *in vivo* germ and somatic cells mutagenicity assay data do not meet the criteria for classification.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the data available for flazasulfuron and according to the criteria under Regulation (EC) No 1272/2008, **no classification of genotoxicity / germ cell mutagenicity** can be derived. **The data is considered conclusive but not sufficient for classification**.

10.9 Carcinogenicity

Table 49: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>Oncogenicity study in rats
(24 months)
Method: US EPA 83-5 and OECD TG 453 (1981)
GLP: Yes
Deviations from current test guideline (OECD TG 453, 2018):
-No satellite groups to monitor the reversibility of toxicological changes were incorporated.
-The recommendation to feed two to four intervals for dose level spacing in low</td>
<td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.3% purity

Oral (diet)

Doses:
Males: 0, 40, 400 and 2000 ppm, equivalent to 0, 1.313, 13.26 and 70.1 mg/kg bw/day.
Females: 0, 40, 400 and 4000 ppm, equivalent to 0, 1.601, 16.45 and 172.6 mg/kg bw/day.

104-weeks feed exposure.</td>
<td>Mortality: No treatment-related differences were reported in mortality for females at any dose level compared with control group. At 2000 ppm male group, an increased mortality from week 72 was observed. All males in this group were terminated in extremis or found dead by week 93.

Cumulative number of animals found dead or killed in extremis during the treatment period.
<table>
<tr><th rowspan="2">Dose (ppm)</th><th colspan="6">Week</th></tr>
<tr><th>52</th><th>62</th><th>72</th><th>82</th><th>93</th><th>104</th></tr>
<tr><th colspan="7">Males (out of 50 animals)</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>2</td><td>6</td></tr>
<tr><td>40</td><td>0</td><td>0</td><td>1</td><td>1</td><td>6</td><td>14* (ndr)</td></tr>
<tr><td>400</td><td>0</td><td>0</td><td>0</td><td>1</td><td>6</td><td>11</td></tr>
<tr><td>2000</td><td>0</td><td>1</td><td>5*</td><td>36*</td><td>50*</td><td>-</td></tr>
<tr><th colspan="7">Females (out of 50 animals)</th></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>3</td><td>14</td></tr>
<tr><td>40</td><td>0</td><td>0</td><td>1</td><td>2</td><td>3</td><td>10</td></tr>
<tr><td>400</td><td>0</td><td>1</td><td>1</td><td>3</td><td>3</td><td>7</td></tr>
<tr><td>4000</td><td>0</td><td>2</td><td>2</td><td>4</td><td>7</td><td>13</td></tr>
</table>
Clinical signs:

In 2000 ppm males with the weeks of treatment in which effects were seen
▪ Emaciation: 44/50 animals (88%) on weeks 67-83 vs 2/50 in controls.
▪ Decreased motor activity: 42/50 animals (84%) on week 66-93 vs 6/50 in controls mainly observed only in one week/animal but in 4 animals</td>
<td>Anonymous 20 (1995a) (CA) B.6.5.1</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
and medium dosage levels were not conducted. Food efficiency was measured only during the first 13 weeks. -Water consumption was not measured. -Prothrombin time and activated partial thromboplastin were not measured at haematology analysis. -Bilirubin parameter is not measured in urinalysis. -Organ weight of epididymides, heart, thyroid, ovaries and uterus were not measured. Rats, SPF Fisher rats (F344/DuCrj) Males and females 85/sex/dose group (50 animals for the main group and 35 animals for the satellite group). HCD for neoplastic lesions in studies conducted in same laboratory an same study conditions (termination: 1993-1995) **Study acceptable**		with 3-5 weeks of duration. ▪ Bradypnea: 35/50 animals (70%) vs 6/50 in controls on week 63-91 mainly observed only in one week of treatment. ▪ Pale eye: 34/50 animals (68%) vs 8/50 in controls on week 63-91 mainly observed only in one week of treatment. ▪ Opaque eyes: 27/50 animals (54%) vs 0/50 in controls on week 75-91 mainly observed only in one week of treatment. ▪ Pale-colored skin: 31/50 animals (62%) vs 5/50 in controls on week 72-91 mainly observed only in one week of treatment. In 4000 ppm females ↑ soiled fur on external genital area was seen in 34/50 animals (68%) vs 10/50 in controls seen on week 17-105 with a prolonged duration. **2000 ppm** (70.1 mg/kg bw/day for ♂) / **4000 ppm** (172.6 mg/kg bw/day for ♀) Bodyweight: *(Bodyweight gain not performed).* ▪ (↓) bw in ♂ throughout week 4- 93 (4-28%). ▪ (↓) bw in ♀ throughout week 0-104 (5-16%). Food consumption: ▪ (↓) in ♂ at week 1 (6%) and throughout week 52-88 (6-27%). ▪ (↓) in ♀ through week 1 (10%), 8 (7%), 9 (6%), 10 (7%), 24 (6%), 36(8%), 48 (7%), 68 (8%), 72 (9%), 76 (7%), and 88 (10%). Food efficiency: ▪ (↓) in ♂/♀ (6%/11%). Haematology: *(only statistically significant differences in male group)* ▪ (↓) Ht at week 13 (3%), 52 (10%) and 78 (28%). ▪ (↓) RBC at week 13 (2%), 52 (10%) and 78 (30%). ▪ (↓) Hb at week 52 (9%) and 78 (25%). ▪ (↑) PLT at week 52 (15% ncdr) and 78 (20%, ncdr). ▪ (↑) WBC at week 52 (31% ncdr) and 78 (25%, ncdr). ▪ (↑) Segmented neutrophil at week 52(86% ncdr) and 78 (80%, ncdr). ▪ (↑) MCV at week 78 (4%). ▪ (↑) MCH at week 78 (8%). ▪ (↑) MCHC at week 78 (4%). ▪ (↑) Reticulocytes at week 78 (92%, ncdr). Clinical biochemistry: ▪ (↑) y-glutamyl transpeptidase (GGPT) in ♂ at week 52 (50%) and 78 (67%). ▪ (↑) creatinine (creat) in ♂ at week 52 (30%) and 78 (269%). ▪ (↑) urea nitrogen (BUN) in ♂ at week 52 (68%) and 78 (638%); and in ♀ at week 78 (15%). ▪ (↑) total cholesterol (T.Chol.) in ♂ at week 52 (44%) and 78 (116%). ▪ (↑) calcium (Ca) in ♂ at week 52 (2%) and 78 (13%). ▪ (↑) inorganic phosphorus (P) in ♂ at week 52 (32%) and 78 (142%). ▪ (↑) potassium (K) in ♂ at week 52 (15%) and 78 (29%). ▪ (↓) glutamic pyruvic transaminase (GPT) in ♂ at week 52 (47%, ncdr) and 78 (42%, ncdr); and in ♀ at week 78 (26%, ncdr). ▪ (↓) albumin/globulin ratio (A/G ratio) in ♂ at week 52 (11%) and 78 (13%). ▪ (↑) creatine phosphokinase (CPK) in ♂ at week 78 (79%, ncdr). ▪ (↑) globulin (Glob) in ♂ at week 52 (6%). ▪ (↓) glutamic oxaloacetic transaminase (GOT) in ♂ at week 52 (31%, ncdr). ▪ (↓) alkaline phosphatase (ALP) in ♂ at week 52 (18%, ncdr); and in ♀	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
		at week 78 (19%, ncdr). ▪ (↓) total bilirubin (T.Bil.) in ♂ at week 26 (23%, ncdr); and in ♀ at week 26 (33%, ncdr) and 52 (25%, ncdr). ▪ (↑) albumin in ♂ at week 26 (3%, ndr) and (↓) at week 52 (6%) and 78 (9%). ▪ (↓) glucose in ♀ at week 78 (14%, ncdr). <u>Urinalysis:</u> ▪ (↑) urine volume in ♂ at week 13 (20%), 26 (29%), 52 (284%) and 78 (368%); and in ♀ at week 13 (38%). ▪ (↑) pH in ♂ at week 26 (30%; 3/10 animals vs 0/10 in controls); and in ♀ at week 78 (20%; 2/10 animals vs 0/10 in controls). ▪ (↑) pale coloured urine in ♂ at week 52 (100%; 10/10 animals vs 0/10 in controls) and 78 (100%; 10/10 animals vs 0/10 in controls). ▪ (↓) urine specific gravity in ♂ at week 52 (4%) and 78 (5%). ▪ (↑) sediments (acicular crystalline materials) in ♂ at week 78 (100%; 10/10 animals vs 0/10 in controls). ▪ (↓) protein in ♀ at week 13 (100%; 10/10 animals vs 4/10 in controls). <u>Organ weights:</u> ▪ Kidney: (↑) abs wt in ♂ at week 26 (37%), 52 (128%) and 78 (30%); and in ♀ at week 52 (9%) and 104 (14%). (↑) rel wt in ♂ at week 26 (39%), 52 (139%) and 78 (92%); and in ♀ at week 26 (8%), 52 (17%), 78 (27%) and 104 (32%). ▪ Liver: (↑) rel wt in ♂ at week 26 (10%), 52 (13%) and 78 (39%); and in ♀ at week 52 (10%) and 78 (15%). ▪ Brain: (↑) rel wt in ♂ at week 52 (7%, ndr) and 78 (47%, ndr); and in ♀ at week 26 (7%, ndr), 78 (22%, ndr) and 104 (14%, ndr). ▪ Spleen: (↑) abs wt in ♂ at week 52 (10%). (↑) rel wt in ♂ at week 52 (13%) and 78 (69%). ▪ Adrenal: (↑) rel wt in ♂ at week 52 (10%, ndr) and 78 (80%, ndr). (↑) abs wt in ♂ at week 78 (24%, ndr); and (↓) in ♀ at week 78 (14%, ndr). ▪ Testes: (↑) rel wt at week 52 (7%, ndr) and 78 (48%, ndr). <u>Necropsy</u> Kidney: ▪ (↑) pale in colour in ♂ main group (22%, 11/49 animals vs 0/50 in controls). ▪ (↑) cyst in ♂ main group (12%; 6/49 animals vs 0/50 in controls). ▪ (↑) coarse surface in ♂ main group (100%; 49/49 animals vs 1/50 in controls) and in interim kills at week 52 (100%; 10/10 animals vs 0/10 in controls) and 78 (100%; 10/10 animals vs 0/10 in controls). ▪ (↑) dark in colour in ♀ main group (34%; 17/50 animals vs 2/50 in controls) and in interim kills at week 52 (100%; 10/10 animals vs 0/10 in controls), 78 (80%; 8/10 animals vs 0/10 in controls) and terminal kill at week 104 (40%; 15/37 animals vs 0/36 in controls). ▪ (↑) enlargement in ♂ in interim kills at week 52 (100%; 10/10 animals vs 0/10 in controls). Aorta: ▪ (↑) sclerosis in ♂ main group (39%; 19/49 animals vs 0/50 in controls). Stomach: ▪ (↑) Whitish wall in glandular portion in male main group (20%; 10/49 animals vs 0/50 in controls). Intestine: ▪ (↑) black contents in small intestine in male main group (10%; 5/49 animals vs 0/50 in controls). ▪ (↑) black contents in large intestine in male main group (10%; 5/49	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		animals vs 0/50 in controls). Parathyroid: ▪ (↑) enlargement in ♂ main group (57%; 28/49 animals vs 0/50 in controls). Eye: ▪ (↑) opacity in ♂ main group (43%; 21/49 animals vs 0/50 in controls). <u>Histopathology</u> Non-neoplastic changes: Kidney: ▪ (↑) Early change of chronic nephropathy in ♂ at week 26 (100%; 10/10 animals vs 4/10 in controls). ▪ (↑) chronic nephropathy in ♂ at week 52 (100%; 10/10 animals vs 0/10 in controls), 78 (100%; 10/10 animals vs 0/10 in controls), and main group (100%; 49/49 animals vs 28/50 in controls); and in ♀ at main group (22%; 11/50 animals vs 3/50 in controls). ▪ (↑) hyaline droplets of proximal tubular cells in ♂ at week 26 (100%; 10/10 animals vs 0/10 in controls), 52 (100%; 10/10 animals vs 0/10 in controls) and 78 (100%; 10/10 animals vs 0/10 in controls). ▪ (↑) luminal dilation of proximal tubule in ♂ at week 26 (100%; 10/10 animals vs 0/10 in controls), 52 (100%; 10/10 animals vs 0/10 in controls), 78 (100%; 10/10 animals vs 0/10 in controls) and main group (100%; 49/49 animals vs 1/50 in controls); and in ♀ at week 78 (90%; 9/10 animals vs 0/10 in controls) and in main group (98%; 49/50 animals vs 0/50 in controls). ▪ (↑) brown pigment deposition of proximal tubular cells in ♂ at week 52 (40%; 4/10 animals vs 0/10 in controls), 78 (100%; 10/10 animals vs 0/10 in controls) and main group (87%; 43/49 animals vs 0/50 in controls); and in ♀ main group (58%; 29/50 animals vs 2/50 in controls). ▪ (↑) pyelic epithelial hyperplasia in ♂ at week 78 (100%; 10/10 animals vs 0/10 in controls) and in the main group (84%; 41/49 animals vs 0/50 in controls). ▪ (↑) mineralization in ♂ main group (77%; 38/49 animals vs 0/50 in controls). Eye: ▪ (↑) keratitis in male main group (18%; 9/49 animals vs 0/50 in controls). ▪ (↑) mineralization of cornea in ♂ main group (22%; 11/49 animals vs 2/50 in controls). ▪ (↑) iritis in ♂ main group (14%; 7/49 animals vs 0/50 in controls). Other organs: ▪ (↑) arterial wall mineralization in the heart of ♂ main group (45%; 22/49 animals vs 0/50 in controls). ▪ (↑) aorta mineralization in ♂ main group (75%, 37/49 animals vs 0/50 in controls, ncdr). ▪ (↑) increased brown pigment deposition in spleen of ♂ main group (84%; 41/49 animals vs 5/50 in controls). ▪ (↑) alveolar wall mineralization in the lung of ♂ main group (51%; 25/49 animals vs 0/50 in controls). ▪ (↑) non glandular and glandular mineralization in the stomach of ♂ main group (16%; 8/49 vs 0/50 in controls and 75%; 37/49 animals vs 0/50 in controls, respectively). ▪ (↑) glandular/submucosal edema in the stomach of ♂ main group (22%; 11/49 animals vs 0/50 in controls). ▪ (↑) erosion/ulcer in large intestine of ♂ main group (16%; 8/49 animals vs 0/50 in controls). ▪ (↑) hyperplasia in parathyroid of ♂ main group (89%; 42/47 animals	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		vs 0/50 in controls). ▪ (↑) sternum/fibrous osteodystrophy in ♂ main group (81%; 40/49 animals vs 0/50 in controls). ▪ (↑) femur/fibrous osteodystrophy in ♂ main group (81%; 40/49 animals vs 0/50 in controls). **400 ppm** (13.26 ♂/16.45 ♀ mg/kg bw/day) Bodyweight: *(bodyweight gain not performed).* ▪ (↓) bw ♂ at weeks 24 (3%, ndr), 68 (3%, ndr), 72 (3%, ndr), 80- 96 (4-5%, ndr) and 104 (5%, ndr). ▪ (↓) bw ♀ at weeks 40 (3%, ndr), 48 (3%, ndr) and 52 (4%, ndr). Haematology: ▪ (↓) Hb in ♂ at week 52 (2%). Clinical biochemistry: *(only statistically significant differences in male group)* ▪ (↑) alkaline phosphatase (ALP) at week 26 (8%, ncdr). ▪ (↑) y-glutamyl transpeptidase (GGPT) at week 104 (33%). ▪ (↑) creatine (Creat.) at week 104 (9%). ▪ (↑) urea nitrogen (BUN) at week 104 (28%). ▪ (↑) total cholesterol (T.Chol.) at week 104 (35%). ▪ (↓) sodium (Na) at week 104 (0.4%). Urinalysis: ▪ (↑) sediments (Acicular crystalline materials) in ♂ at week 78 (40%; 4/10 animals vs 0/10 in controls) and 104 (80%; 8/10 animals vs 1/10 in controls). ▪ (↑) urine volume in ♂ at week 104 (60%), ▪ (↑) protein in ♀ at week 26 (10%; 1/10 animals vs 0/10 in controls, ndr). Organ weights: ▪ Kidney: (↑) abs wt in ♂ at week 52 (16%), 78 (25%), and 104 (27%). (↑) rel wt in ♂ at week 52 (18%), 78 (35%) and 104 (28%), ▪ Liver: (↑) rel wt in ♂ at week 26 (6%), 52 (7%) and 104 (10%). Necropsy Kidney: ▪ (↑) dark in colour in ♂ main group (64%; 32/50 animals vs 4/50 in controls). ▪ (↑) coarse surface in ♂ main group (28%; 14/50 animals vs 1/50 in controls). ▪ (↑) enlargement in ♂ in interim kills at week 78 (100%; 10/10 animals vs 0/10 in controls). Histopathology Non-neoplastic changes: Kidney: ▪ (↑) Early change of chronic nephropathy in ♂ at week 52 (100%, 10/10 animals vs 3/10 in controls). ▪ (↑) chronic nephropathy in ♂ at week 78 (40%, 4/10 animals vs 0/100 in controls) and main group (94%, 47/50 animals vs 28/50 in controls). ▪ (↑) hyaline droplets of proximal tubular cells in ♂ at week 26 (70%; 7/10 animals vs 0/10 in controls), 52 (100%; 10/10 animals vs 0/10 in controls) and 78 (100%; 10/10 animals vs 0/10 in controls). ▪ (↑) pyelic epithelial hyperplasia in ♂ main group (22%; 11/50 animals vs 0/50 in controls; n.s.). ▪ (↑) luminal dilation of proximal tubule in ♂ at week 52 (100%; 10/10 animals vs 0/10 in controls), 78 (100%; 10/10 animals vs 0/10 in controls) and main group (100%; 50/50 animals vs 1/50 in controls).	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>▪ (↑) brown pigment deposition of proximal tubular cells in ♂ main group (36%; 18/50 animals vs 0/50 in controls).

40 ppm (1.313 ♂/1.601 ♀ mg/kg bw/day)
<u>Clinical biochemistry:</u>
▪ (↑) alkaline phosphatase (ALP) in ♂ at week 26 (8%, ncdr).
▪ (↓) glutamic oxaloacetic transaminase (GOT) in ♂ at week 52 (25%, ncdr).
▪ (↓) glucose in ♀ at week 78 (11%, ndr).

NOAEL_{toxicity}: 40 ppm corresponding to 1.313/ 1.601 mg/kg bw/day for ♂/♀, respectively.
NOAEL_{carcinogenicity}: >2000/4000 ppm corresponding to >70.1/ 172.6 mg/kg bw/day for ♂/♀, respectively.</td><td></td></tr>
<tr><td>Oncogenicity study in mice

(18 months)

<u>Method:</u> US EPA 83-2

GLP: Yes

<u>Deviations from current test guideline (OECD TG 453, 2018):</u>

-No satellite groups to monitor the reversibility of toxicological changes were incorporated.

-Age at study start (39 days weeks) older than recommended (as soon as possible after weaning; 21 days in mice).

-The recommendation to feed two to four intervals for dose level spacing in low and medium dosage levels were not conducted.

-Food efficiency was not measured.

-Organ weight of epididymides, heart, ovaries, spleen, thyroid and uterus were</td><td><u>Test substance:</u>
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.3% purity

Oral (diet)

Doses: 0, 500, 3500 and 7000 ppm, equivalent to:
Males: 0, 70.4, 497, 8 and 987.4 mg/kg bw/day.
Females: 0, 88.5, 596.4 and 1165.5 mg/kg bw/day.

78-weeks feed exposure.</td><td><u>Mortality</u>: No treatment-related differences were observed in mortality in both sexes at any dose level at the end of the study. However, a slight increase in mortality was detected in all dose male groups between weeks 52-65.

Cumulative of spontaneous and moribund deaths.

<table>
<tr><th rowspan="2">Dose (ppm)</th><th colspan="6">Week</th></tr>
<tr><th>13</th><th>26</th><th>39</th><th>52</th><th>65</th><th>80</th></tr>
<tr><td colspan="7">Males (out of 60 animals)</td></tr>
<tr><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>8</td></tr>
<tr><td>500</td><td>0</td><td>0</td><td>1</td><td>4</td><td>6</td><td>10</td></tr>
<tr><td>3500</td><td>1</td><td>1</td><td>2</td><td>2</td><td>6</td><td>15</td></tr>
<tr><td>7000</td><td>0</td><td>0</td><td>1</td><td>2</td><td>6</td><td>10</td></tr>
<tr><td colspan="7">Females (out of 60 animals)</td></tr>
<tr><td>0</td><td>0</td><td>1</td><td>2</td><td>5</td><td>7</td><td>9</td></tr>
<tr><td>500</td><td>1</td><td>1</td><td>2</td><td>3</td><td>6</td><td>10</td></tr>
<tr><td>3500</td><td>0</td><td>1</td><td>1</td><td>1</td><td>4</td><td>15</td></tr>
<tr><td>7000</td><td>0</td><td>2</td><td>2</td><td>2</td><td>5</td><td>15</td></tr>
</table>

<u>Clinical signs</u>: No treatment related clinical signs were observed.

7000 ppm (987.4 ♂/ 1165.5 ♀ mg/kg bw/day)
<u>Bodyweight and bodyweight gain:</u>
▪ (↓) bw in ♂ throughout weeks 1-77 (4-7%, ncdr).
▪ (↓) bwg in ♂ throughout weeks 0-12 (17%, ncdr).
▪ (↓) bw in ♀ throughout weeks 1-77 (4-6%).
▪ (↓) bwg in ♀ throughout weeks 1-77 (14%).

<u>Food consumption:</u>
(Food efficiency not measured).
▪ (↓) abs food consumption in ♀ throughout weeks 1-77 (6-18%).
▪ (↓) rel food consumption in ♀ throughout weeks 1-77 (5-13%).

<u>Haematology:</u>
▪ (↓) monocytes at 12 months in ♂ (24%, ndr).
▪ (↑) monocytes at 18 months in ♂ (73%, ndr).
▪ (↑) eosinophils at 12 and 18 months in ♂ (135% and 73%) and ♀ (68% and 76%).

<u>Organ weights:</u>
▪ Liver: (↑) abs and rel wt in ♂ (24% and 23%) and ♀ (24% and 33%).

<u>Histopathology:</u>
Non-neoplastic changes (statistical analysis not performed):
▪ (↑) incidence of hepatocellular hypertrophy in ♂ (92%; 55/60 animals vs 11/60 in controls) and ♀ (67%; 40/60 animals vs 10/60 in controls).
▪ (↑) incidence of hepatocellular pigment in ♂ (15%; 9/60 animals vs 2/60</td><td>Anonymous 21 (1995a) (CA) B.6.5.2</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td>not measured.
-Haematology analysis was only performed at 12 months and at termination study (18 months), instead of 3 and 6 months.
-Haematology analysis only included leukocyte determinations.
-Clinical biochemistry and urinalysis were not performed.
Ophthalmological examination was not performed.
-Statistical analysis not performed in non-neoplastic findings.
Mice, Crl:CD-1 (ICR) BR VAF/PLUS® mice
Males and females 60/sex/dose group
Study acceptable</td><td></td><td>in controls).
<u>Incidence of Hepatocellular Hypertrophy</u>
<table>
<tr><th></th><th colspan="3">0 ppm</th><th colspan="3">500 ppm</th><th colspan="3">3500 ppm</th><th colspan="3">7000 ppm</th></tr>
<tr><th></th><th>SS</th><th>USD</th><th>Total</th><th>SS</th><th>USD</th><th>Total</th><th>SS</th><th>USD</th><th>Total</th><th>SS</th><th>USD</th><th>Total</th></tr>
<tr><td>Males</td><td>8</td><td>3</td><td>11</td><td>15</td><td>1</td><td>16</td><td>33</td><td>8</td><td>41</td><td>45</td><td>10</td><td>55</td></tr>
<tr><td>Females</td><td>6</td><td>4</td><td>10</td><td>5</td><td>1</td><td>6</td><td>22</td><td>6</td><td>28</td><td>31</td><td>9</td><td>40</td></tr>
<tr><td>Total</td><td>14</td><td>7</td><td>21</td><td>20</td><td>2</td><td>22</td><td>55</td><td>14</td><td>69</td><td>76</td><td>19</td><td>95</td></tr>
</table>
SS = scheduled sacrifice
USD = unscheduled sacrifice and death

3500 ppm (497.8/ 596.4 mg/kg bw/day for ♂/♀)
<u>Bodyweight and bodyweight gain:</u>
▪ (↓) bw in ♂ from throughout weeks 2-25 (4%, ncdr).
▪ (↓) bw in ♀ throughout weeks 1-73 (5%).
▪ (↓) bwg in ♀ throughout weeks 1-77 (8-52%).

<u>Food consumption:</u>
▪ (↓) abs food consumption in ♀ throughout weeks 1-77 (7-8%).
▪ (↓) rel food consumption in ♀ throughout weeks 1-77 (7-8%).

<u>Haematology:</u>
▪ (↑) lymphocytes at 12 months in ♂ (13%, ncdr).

<u>Organ weights:</u>
▪ Liver: (↑) abs and rel wt in ♂ (22% and 21%) and rel wt in ♀ (19%).

<u>Histopathology:</u>
▪ (↑) incidence of hepatocellular hypertrophy in ♂ (68%; 41/60 animals vs 11/60 in controls) and ♀ (47%; 28/60 animals vs 10/60 in controls).
▪ (↑) incidence of hepatocellular pigment in ♂ (8%; 5/60 animals vs 2/60 in controls).

500 ppm (70.4 ♂/ 88.5 ♀ mg/kg bw/day)
<u>Bodyweight and bodyweight gain:</u>
▪ (↓) bwg in ♀ at week 1 (23%), 49 (8%), 53 (12%), 65 (12%), 69 (10%) and 73 (12%).

NOAEL_{toxicity}: 500 ppm corresponding to 70.4/ 88.5 mg/kg bw/day for ♂/♀, respectively.

NOAEL_{carcinogenicity}: >7000 ppm corresponding to 987.4/ 1165.5 mg/kg bw/day for ♂/♀, respectively.</td><td></td></tr>
</table>

Table 50: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 51: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Three chronic toxicity/carcinogenicity studies were provided with flazasulfuron, one in rats (Anonymous 20, 1995a), one in mice (Anonymous 21, 1995a) and one in dogs (Anonymous 22, 1995b).

In the 24 months oncogenicity study in rats (Anonymous 20, 1995a; B.6.5.1), flazasulfuron was tested at dose levels 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.

The rationale for selection of dose levels arises from a previous 13-week oral subchronic toxicity study in rats (Anonymous 41, 1988b; B.6.3.2.1), in which dietary levels of 0, 40, 200, 1000, and 5000 ppm were used in 12 animals for each sex and dose group. In the 5000 ppm group, both males and females exhibited reduced bodyweights as compared with the controls, and males showed a decrease in food efficiency. In addition, necropsy examination of males revealed tubular atrophy and dilation of proximal tubules of the kidneys in all animals treated with the high dose. In the 1000 ppm dose group, males showed a slight depressed bodyweight and an increase in relative weight of the liver. In 1000 ppm females group, together with males and females in 200 ppm groups or less, no treatment-related abnormalities were observed. Based on these results, the definitive doses of 0, 40, 400 and 2000/4000 (males/females) ppm were selected for the main oncogenicity study.

In the 2-year study, no mortality or morbidity signs were observed in females at any dose level. On the other hand, males in the high dose group showed a statistically significant increased mortality during the period later than week 72, and all of them were killed in extremis or found dead before week 94. Histopathological examinations revealed that the increased mortality was related to the severe renal failure ascribed to the treatment. In the low dose group, males showed significantly increased mortality (28%; 14/50 animals, vs. 12%; 6/50 animals in the control group) at week 104, however, there were no histopathological lesions or clinical observations considered to be related to treatment.

Table 52: Cumulative mortality for the main group

Dose (ppm)	Week					
	52	**62**	**72**	**82**	**93**	**104**
Males (out of 50 animals)						
0	0	0	0	1	2	6
40	0	0	1	1	6	14
400	0	0	0	1	6	11
2000	0	1	5	36	50	-
Females (out of 50 animals)						
0	0	0	0	1	3	14
40	0	0	1	2	3	10
400	0	1	1	3	3	7
4000	0	2	2	4	7	13

For males at 2000 ppm group, a statistically significant increase in incidences of emaciation, decreased motor activity, bradypnea, pale and opaque eyes, and pale-coloured skin was observed. For females at 4000 ppm group, a statistically significant increase in hair loss and soiled fur on external genital area was observed, but only soiled fur was associated to treatment, because of an increase of urine volume was observed in this group. No treatment-related differences in incidence of clinical signs between the control and the treated groups were detected for both sexes at 400 ppm or lower doses.

Statistically significant decrease in bodyweight was recorded in the high dose male group from week 4 to week 93 (4-28%). This observation was attributed to the treatment because decreased food consumption and food efficiency were associated. Females in the 4000 ppm treated group exhibited statistically significantly lower

bodyweight throughout the treatment period (5-16%), along with declined food consumption and food efficiency. Males and females in the mid dose group showed lowered bodyweights at different points throughout the treatment period. Although this change was not associated with depression in food consumption or food efficiency, the plausibility of its toxicological significance was conceived on account of occasional evidences of statistically significantly lowered bodyweights during the treatment period.

Food consumption at 4000 ppm female group, was significantly lower than that of the control group during the treatment period, and group mean food consumption was reduced a 5% compared with control average. In the 2000 ppm male group, food consumption was significantly lower than that of the control at week 1 (6%), and from week 52 to 88 (6-27%). Group mean food consumption during the treatment was decreased a 9% compared with control average. There were no treatment-related differences in mean food consumption for both sexes at 400 ppm and lower doses. Regarding food efficiency measured during first 13 weeks, males and females of high dose groups, showed a 6% and 11% lower values than control group, respectively. In the 400 and 40 ppm treated groups, there were no statistically significant differences in food efficiency as compared with the respective control of either sex.

No ophthalmologic alterations were observed in male and females at high dose groups.

Haematology analysis revealed decreased values of hematocrit (Ht), erythrocyte count (RBC) or hemoglobin (Hb), and an increased value of reticulocyte in males of the 2000 ppm group. Taken together, these results indicate anemia as toxic effect related- treatment. A decreased value in Hb was also detected at 400 ppm male group in the 52 week measure point, indicating a slight presence of anemia. Furthermore, haematology data of males in the high dose group revealed increased values of mean corpuscular volume (MCV), indicating macroerythrocytosis; and an increase of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) after 78 weeks of treatment, which might be a secondary change attributed to the severe decrease of erythrocytes. The toxicological implication of other haematological parameters with significant deviations remains unclear. There were no treatment-related changes in females at any dose level or in males at 40 ppm.

Clinical biochemistry showed significant differences that were mostly detected in males. Analysis revealed statistically significant increased values in creatinine (Creat) in high dose group at week 52 (30%) and 78 (269%), and in mid dose group at week 104 (9%); increased values in blood urea nitrogen (BUN) in 2000 ppm dose level at week 52 (68%) and 78 (638%), and in 400 ppm group at week 104 (28%); and increased values in total cholesterol (T.chol) in high dose group at week 52 (44%) and 78 (116%), and in mid dose group at week 104 (35%). Besides, increased values in calcium (Ca), phosphorous (P) and potassium (K) at week 52 and 78 were noted in 2000 ppm group, along with decreased sodium (Na) value at week 104 (0.4%) in 400 ppm group. Taken together, these findings were consequence of severe renal toxicity of the test substance.

Moreover, increased values of y-glutamyl transpeptidase (GGTP) were detected in 2000 ppm male group at week 52 (50%) and 78 (67%), and in 400 ppm male group at week 104 (33%). These changes might suggest some hepatotoxicity of the test substance, and are in line with increased relative liver weight observed in males of these groups. In addition, high dose male group exhibited decreased values of albumin (Alb) at week 52 (6%) and 78 (9%), and albumin/globulin ratio (A/G ratio) at week 52 (11%) and 78 (13%), as well as increased value of globulin (Glob) after 52 (6%) weeks of treatment. On the whole, these might suggest an impairment of protein metabolism as a consequence of treatment. Other changes recorded in clinical biochemistry were considered to be of no toxicological significance or incidental nature due to were non dose-related.

Urinalysis confirms the renal toxicity of the test substance showing an increase of urine volume in high dose male group in all measure points (20-368%), in 4000 ppm female group at week 13 (38%), and in 400 ppm male group at termination (60%). Moreover, high dose male group showed decreased specific gravity at week 52 (4%) and 78 (5%); increased incidences of pale colored urine at week 52 (100%) and 78 (100%); and an increase of elevated pH observations at week 26 (30%). High dose female group also exhibited elevated pH incidences at week 78 (20%), together with a decreased protein value at week 13 (100%). Additionally, acicular crystalline materials were also detected in males in high dose group at week 78 (100%), and in mid dose group at week 78 (40%) and 104 (80%). This observation might be a symptom of the spontaneous chronic nephropathy which is one of the major senile changes in male rats. On the other hand, females didn’t show

crystals in urine sediments, suggesting a difference between males and females in response to the treatment of the test substance in urinalysis.

Regarding organ weights, males and females treated at high dose, together with mid dose males, exhibited an increase of absolute and relative kidney weights, and an increase of relative liver weight. These changes were dose-related. Absolute and/or relative weights of the spleen noted in 2000 ppm male group were attributable to increase of brown pigment deposition observed in males killed in *extremis* or found dead and overall. The changes were considered to be treatment-related. Other variations noted in weights of brain, adrenal and testes were considered to be secondary changes as consequence of the depression of bodyweight gain or to be of incidental nature due to no supportive evidences were observed histopathologically.

Most important necropsy findings showed renal lesions and were further supported by histopathological analysis. These observations include an increase of coarse surface and enlargement of kidney in males of mid and high dose groups; dark in color in females of the high dose group and males of the mid dose group; pale in color and the presence of cysts in males of the 2000 ppm group. Incidences in other organs were also observed in 2000 ppm male group as sclerosis of the aorta, whitish wall in glandular portion of the stomach, enlargement of the parathyroid, and opacity of the eye; all of them were subsequently confirmed in the histopathological analysis. In addition, males of the high dose group showed increased incidences of emaciation and black contents in the intestine, which were considered to be related to debility caused by the renal failure.

Histopathological examinations in males revealed severe renal failure and confirms previous findings. Analyses displayed evidences of increased incidences of early change of chronic nephropathy in 2000 ppm group at week 26 (100%), and in 400 ppm group at week 52 (100%); increased chronic nephropathy in 100% of high dose animals necropsied at week 52, 78 and main group, and in 400 ppm group at week 78 (40%) and at terminal sacrifice (94%); increased hyaline droplet deposition of proximal tubular cells in 100% of 2000 ppm males necropsied at week 26, 52, 78 and main group, and in mid dose group at week 26 (70%), 52 (100%), 78 (100%) and main group (100%); increased brown pigment deposition in proximal tubular cell in high dose group at week 52 (40%), 78 (100%) and at terminal sacrifice (87%), and in mid dose males at main group (36%); increased luminal dilation of proximal tubule in 100% of high dose group necropsied at week 26, 52, 78 and main group, and in 100% of mid dose males necropsied at week 52, 78 and main group; increased pelvic epithelial hyperplasia of the kidney at week 78 (100%) and at terminal sacrifice in high dose group (84%), and a slightly trend in mid dose group (22%); and increased mineralization in high dose main group (77%).

It is important to notice that the major carrier protein for chemicals in blood in male rats is α2μ-globulin. When the carrier protein is bind to particular class of chemicals, it sometimes produces special complexes which are very resistant to catabolic attack by proteolytic enzymes. During the excretion process of these complexes, they are engulfed by lysosomes in proximal tubular cells in the kidney. However, due to their resistibility to catabolic attack by lysosomal enzymes, they become to deposit in lysosomes, to be conglomerated each other and develop a large molecular structure in the cytoplasm. When these structures occupy a large part of the cytoplasm, an abundant distribution of hyaline droplets on microscope is observed, and the integrity and function of tubular cells become then compromised leading to renal dysfunction. Among these particular of chemicals, the development of renal toxicity has been well studied in Limonene and Unleaded gasoline which induce renal failure in male rats associated with vigorous deposition of hyaline droplets in proximal tubular cells that are positive to immunohistochemistry for α2μ-globulin.

In this study, it is probably that the severe renal failure that causes early deaths in 2000 ppm male group might be classified as α2μ-globulin nephropathy. In order to demonstrate the hypothesis, a 2-week oral (gavage) study (Anonymous 46, 1995; B.6.8.2.3) with the test substance was carried out in male rats at a dose level of 0, 400, and 800 mg/kg bw/day, and immunohistological staining to α2μ-globulin was conducted on proximal tubular cells in the kidney. The results showed cytoplasmic granules which were widely distributed along the cytoplasm and consistent to hyaline droplets on hematoxylin-eosin specimen. Based on these results, hyaline droplets in proximal tubular cells in males of the 2000 and 400 ppm groups were strongly suggested as α2μ-globulin. Therefore, the induced renal lesions such as chronic nephropathy, dilation of proximal tubule, increased brown pigment deposition in proximal tubular cells and pelvic epithelial hyperplasia were considered to be expression of α2μ-globulin nephropathy. The production of α2μ-globulin in the liver of F344 male rat

is reported to increase with aging until reaching a plateau by the age of 100 days. In the previous 13-week subchronic toxicity study (Anonymous 41, 1988b; B.6.3.2.1), authors did not detect hyaline droplets in tubular cells and it was considered that the hyaline droplet deposition had not been formed at termination of the subchronic study because production of α2μ-globulin in the liver was still insufficient to produce an amount of hyaline droplets in tubular cells. It was assumed that, at termination of 13-weeks subchronic study, tubular cells could still keep a certain level of proteolytic catabolism enough to maintain the physiological integrity of the cell. When the test substance was administered by incorporation into diet, it would take long time to produce hyaline droplets observable microscopically.

Accordingly, there is evidence that mechanism by which chemicals induced excessive accumulation of α2μ-globulin (CIGA) is not relevant for human risk assessment[1][2][3]. α2μ-Globulin is a male rat-specific protein, and hyaline droplet accumulation or the spectrum of lesions comprising α2μ-globulin nephropathy has not been observed in female rats or mice of either sex following treatment with CIGA. In contrast, chronic progressive nephropathy induced by α2μ-globulin accumulation is a renal dysfunction that does not occur in humans, and it is known that renal injury induced by chemicals involved the glomerulus in humans, instead of renal tubular cells. Thus, the weight of evidence (WoE) supports an absence of a renal counterpart in humans. In addition, humans do not have a protein with a function comparable to α2μ-globulin, again on a qualitative basis, there is not concordance for this particular key event between rats and humans, and therefore, this MoA is qualitatively not relevant to humans.

On the other hand, males in the high dose group exhibited increased incidences of hyperplasia of parathyroid, mineralization of the arterial wall of the heart, non-glandular and glandular mineralization portion of the stomach, submucosal edema of the stomach, ulcer of the large intestine, alveolar wall mineralization of the lung and cornea, keratitis and iritis of the eye, and fibrous osteodystrophy of the sternum and femur bone. Taken together, these observations were considered to be related to the secondary hyperparathyroidism following to severe chronic nephropathy. Most of the brown pigments increased in the spleen of males in the 2000 ppm were identified as hemosiderin, and considered to be attributed to anemia observed in this group.

Histopathological kidney examinations of 4000 ppm female group showed signs of chronic nephropathy (22%) at terminal sacrifice, but in a lower incidence that than observed in males at 2000 ppm (100%). In addition, signs of renal failure as an increase of luminal dilation of proximal tubule and brown pigment deposition in proximal tubular cells were recorded (98% and 58%, respectively) in high dose female group. However, no hyaline droplets depositions in proximal tubular cells, pyelic epithelial hyperplasia or mineralization incidences were detected in 4000 ppm females. Accordingly, it was considered that the renal failure observed in females of the highest dose group was developed through a mechanism different from α2μ-globulin nephropathy, and there was a sex difference in response to the treatment of the test substance.

Neoplastic alterations observed were considered not related to the treatment, due to the comparable incidence between the treated and control group animals (Table 53 to Table 60). The only increase seen was in interstitial cell tumour in the testis (Table 61) observed in males but the incidence for all animals was also comparable to historical control data (HCD) in control male Fischer (F344/DuCrj) rats in Institute of Environmental Toxicology (IET) studies conducted in same study conditions with SL-160 technical (termination: 1993-1995).

[1] Hard GC, Rodgers IS, Baetcke KP, Richards WL, McGaughy RE, Valcovic LR. (1993). Hazard evaluation of chemicals that cause accumulation of α2μ-globulin, hyaline droplet nephropathy, and tubule neoplasia in the kidneys of male rats. *Environ. Health Perspect.* **99**:313-49.

[2] Hard GC, and Khan KN. (2004). A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. *Toxicol. Pathol.* **32:** 171–180.

[3] Hard GC. et al. (2012). Consideration of rat chronic progressive nephropathy in regulatory evaluations for carcinogenicity. *Toxicol. Sci.* **132**(2):268-75.

Table 53: Neoplastic lesions in male rats (satellite group) after 52 weeks of treatment

Site & Lesion	Dose (ppm)	0	40	400	2000
	No. of animals examined	10	10	10	10
Respiratory System					
Lung:	(N-)	(10)	(10)	(10)	(10)
(M) Adenocarcinoma		1	0	0	0
Endocrine System					
Pituitary:	(N-)	(10)	(0)	(1)	(10)
(B) Anterior adenoma		1	-	1	0
Thyroid:	(N-)	(10)	(0)	(0)	(10)
(B) C-cell adenoma		1	-	-	0

Malignancy: (B) Benign neoplams (M) Malignant neoplasms

* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 54: Neoplastic lesions in male rats (main group) after terminal kill after 104 weeks of treatment

Site & Lesion	Dose (ppm)	0	40	400	2000
	No. of animals examined	44	36	39	0
Cardiovascular System					
Heart:	(N-)	(44)	(0)	(39)	(0)
(B) Schwannoma		3	–	1	–
(M) Malignant schwannoma		1	–	0	–
Hematopoietic & Lymphatic System					
General:	(N-)	(44)	(36)	(39)	(0)
(M) Mononuclear cell leukemia		2	1	4	–
Bone marrow (femur):	(N-)	(44)	(0)	(39)	(0)
(M) Histiocytic sarcoma		0	–	1	–
Thymus:	(N-)	(44)	(0)	(39)	(0)
(M) Malignant thymoma		0	–	1	–
Spleen:	(N-)	(44)	(2)	(39)	(0)
(M) Hemangiosarcoma		1	0	0	–
(M) Histiocytic sarcoma		1	0	0	–
Respiratory System					
Lung:	(N-)	(44)	(36)	(39)	(0)
(B) Adenoma		4	6	3	–
Digestive System					
Small intestine:	(N-)	(44)	(0)	(39)	(0)
(B) Adenoma		1	–	0	–
Liver:	(N-)	(44)	(36)	(39)	(0)
(B) Hepatocellular adenoma		5	0*	0*	–
(M) Hepatocellular carcinoma		0	1	0	–
Pancreas:	(N-)	(44)	(1)	(39)	(0)
(B) Islet cell adenoma		6	1	1	–
Urinary System					
Kidney:	(N-)	(44)	(36)	(39)	(0)
(B) Lipoma		0	0	1	–
Genital System					
Testis:	(N-)	(44)	(36)	(39)	(0)
(B) Interstitial cell tumor		42	36	36	–
Preputial gland:	(N-)	(4)	(1)	(3)	(0)
(B) Adenoma		3	1	2	–
(M) Carcinoma		1	0	0	–
Endocrine System					
Pituitary:	(N-)	(44)	(5)	(39)	(0)
(B) Anterior adenoma		4	4	9	–
(B) Adenoma in intermediate part		1	0	0	–
Thyroid:	(N-)	(44)	(8)	(39)	(0)
(B) C-cell adenoma		13	4	6	–
(M) C-cell carcinoma		0	0	1	–
Adrenal:	(N-)	(44)	(3)	(39)	(0)
(B) Pheochromocytoma		8	3	8	–
Nervous System					
Cerebrum:	(N-)	(44)	(0)	(39)	(0)
(B) Glioma		0	–	1	–
(B) Granular cell tumor		1	–	0	–
Integumentary System					
Skin:	(N-)	(44)	(13)	(39)	(0)
(B) Papilloma		3	1	2	–
— Integumentary System 《cont. 》					
Skin 《cont. 》:	(N-)	(44)	(13)	(39)	(0)
(B) Keratoacanthoma		3	1	1	–
(B) Fibroma		4	3	3	–
(B) Lipoma		1	2	2	–
(M) Squamous cell carcinoma		1	1	2	–
(M) Fibrosarcoma		0	0	1	–
< Nodule/mass not in section >		1	0	0	–
Mammary gland:	(N-)	(2)	(0)	(1)	(0)
(B) Fibroadenoma		2	–	1	–
Body Cavities					
Abdominal cavity:	(N-)	(4)	(1)	(5)	(0)
(B) Lipoma		0	0	1	–
(M) Malignant mesothelioma		2	0	1	–

Malignancy: (B) Benign neoplams (M) Malignant neoplasms

* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 55: Neoplastic lesions in male rats (main group) of animals killed in extremis or found dead

Site & Lesion	Dose (ppm)	0	40	400	2000
	No. of animals examined	6	14	11	49
Hematopoietic & Lymphatic System					
General:	(N-)	(6)	(14)	(11)	(49)
(M) Mononuclear cell leukemia		3	8	2	3*
Respiratory System					
Lung:	(N-)	(6)	(14)	(11)	(49)
(B) Adenoma		1	1	0	0
(M) Squamous cell carcinoma		0	1	0	0
(M) Adenocarcinoma		0	0	1	0
Digestive System					
Small intestine:	(N-)	(6)	(14)	(11)	(49)
(M) Adenocarcinoma		0	1	0	0
(M) Leiomyosarcoma		0	1	0	0
Liver:	(N-)	(6)	(14)	(11)	(49)
(B) Hepatocellular adenoma		1	0	0	1
Pancreas:	(N-)	(6)	(14)	(11)	(49)
(B) Islet cell adenoma		1	1	1	0
Urinary System					
Kidney:	(N-)	(6)	(14)	(11)	(49)
(M) Transitional cell carcinoma		0	0	1	0
(M) Liposarcoma		0	0	1	0
Genital System					
Testis:	(N-)	(6)	(14)	(11)	(49)
(B) Interstitial cell tumor		1	10*	8*	46**
Preputial gland:	(N-)	(0)	(5)	(1)	(2)
(B) Adenoma		–	2	1	1
(M) Squamous cell carcinoma		–	0	0	1
(M) Carcinoma		–	1	0	0
Endocrine System					
Pituitary:	(N-)	(6)	(14)	(11)	(49)
(B) Anterior adenoma		3	5	3	3*
Thyroid:	(N-)	(6)	(14)	(11)	(49)
(B) C-cell adenoma		1	2	0	3
(M) C-cell carcinoma		0	0	1	1
Adrenal:	(N-)	(6)	(14)	(11)	(49)
(B) Cortical adenoma		1	0	0	0
(B) Pheochromocytoma		2	0	0	2
Sense Organs					
Ear:	(N-)	(0)	(1)	(0)	(0)
(M) Zymbal´s glamd carcinoma		–	1	–	–
Integumentary System					
Skin:	(N-)	(6)	(14)	(11)	(49)
(B) Keratoacanthoma		0	1	0	1
(B) Fibroma		0	3	0	1
Body Cavities					
Thoracic cavity:	(N-)	(0)	(2)	(3)	(0)
(M) Osteosarcoma		–	0	1	–
(M) Malignant mesothelioma		–	1	0	–
Abdominal cavity:	(N-)	(1)	(2)	(0)	(0)
(M) Fibrosarcoma		1	0	–	–
(M) Malignant mesothelioma		0	1	–	–

Malignancy: (B) Benign neoplams (M) Malignant neoplasms

* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 56: Neoplastic lesions in male rats (main group)

Site & Lesion	Dose (ppm)	0	40	400	2000
	No. of animals examined	50	50	50	49
Cardiovascular System					
Heart:	(N-)	(50)	(14)	(50)	(49)
(B) Schwannoma		3	0	1	0
(M) Malignant schwannoma		1	0	0	0
Hematopoietic & Lymphatic System					
General:	(N-)	(50)	(50)	(50)	(49)
(M) Mononuclear cell leukemia		5	9	6	3
Bone marrow(femur):	(N-)	(50)	(13)	(50)	(49)
(M) Histiocytic sarcoma		0	0	1	0
Thymus:	(N-)	(50)	(14)	(50)	(49)
(M) Malignant thymoma		0	0	1	0
Spleen:	(N-)	(50)	(16)	(50)	(49)
(M) Hemangiosarcoma		1	0	0	0
(M) Histiocytic sarcoma		1	0	0	0
Respiratory System					
Lung:	(N-)	(50)	(50)	(50)	(49)
(B) Adenoma		5	7	3	0*
(M) Squamous cell carcinoma		0	1	0	0
(M) Adenocarcinoma		0	0	1	0
Digestive System					
Small intestine:	(N-)	(50)	(14)	(50)	(49)
(B) Adenoma		1	0	0	0
(M) Adenocarcinoma		0	1	0	0
(M) Leiomyosarcoma		0	1	0	0
Liver:	(N-)	(50)	(50)	(50)	(49)
(B) Hepatocellular adenoma		6	0*	0*	1
(M) Hepatocellular carcinoma		0	1	0	0
Pancreas:	(N-)	(50)	(15)	(50)	(49)
(B) Islet cell adenoma		7	2	2	0**
Urinary System					
Kidney:	(N-)	(50)	(50)	(50)	(49)
(B) Lipoma		0	0	1	0
(M) Transitional cell carcinoma		0	0	1	0
(M) Liposarcoma		0	0	1	0
Genital System					
Testis:	(N-)	(50)	(50)	(50)	(49)
(B) Interstitial cell tumor		43	46	44	46
Preputial gland:	(N-)	(4)	(6)	(4)	(2)
(B) Adenoma		3	3	3	1
(M) Squamous cell carcinoma		0	0	0	1
(M) Carcinoma		1	1	0	0
Endocrine System					
Pituitary:	(N-)	(50)	(19)	(50)	(49)
(B) Anterior adenoma		7	9	12	3
(B) Adenoma in intermediate part		1	0	0	0
Thyroid:	(N-)	(50)	(20)	(50)	(49)
(B) C-cell adenoma		14	6	6*	3**
(M) C-cell carcinoma		0	0	2	1
Adrenal:	(N-)	(50)	(17)	(50)	(49)
(B) Cortical adenoma		1	0	0	0
Endocrine System 《cont.》					
Adrenal 《cont.》:	(N-)	(50)	(17)	(50)	(49)
(B) Pheochromocytoma		10	3	8	2*
Nervous System					
Cerebrum:	(N-)	(50)	(14)	(50)	(49)
(B) Glioma		0	0	1	0
(B) Granular cell tumor		1	0	0	0
Sense Organs					
Ear:	(N-)	(0)	(1)	(0)	(0)
(M) Zymbal's glamd carcinoma		–	1	–	–
Integumentary System					
Skin:	(N-)	(50)	(27)	(50)	(49)
(B) Papilloma		3	1	2	0
(B) Keratoacanthoma		3	2	1	1
(B) Fibroma		4	6	3	1
(B) Lipoma		2	2	2	0
(M) Squamous cell carcinoma		2	1	2	1
(M) Fibrosarcoma		0	0	2	0
< Nodule/mass not in section >		1	1	0	0
Mammary gland:	(N-)	(2)	(0)	(2)	(1)
(B) Fibroadenoma		2	–	1	1
(B) Fibroma		0	–	1	0
Body Cavities					
Thoracic cavity:	(N-)	(0)	(2)	(3)	(0)
(M) Osteosarcoma		–	0	1	–
(M) Malignant mesothelioma		–	1	0	–
Abdominal cavity:	(N-)	(5)	(3)	(5)	(0)
(B) Lipoma		0	0	1	–
(M) Fibrosarcoma		1	0	0	–
(M) Malignant mesothelioma		2	1	1	–

Malignancy: (B) Benign neoplams (M) Malignant neoplasms

* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 57: Neoplastic lesions in female rats (satellite group) after 52 weeks of treatment

Site & Lesion	Dose (ppm)	0	40	400	4000
	No. of animals examined	10	10	10	10
Respiratory System					
Lung:	(N-)	(10)	(10)	(10)	(10)
(B) Adenoma		0	0	0	1
Genital System					
Uterine horn:	(N-)	(10)	(0)	(0)	(10)
(B) Endometrial stromal polyp		0	–	–	1
Endocrine System					
Pituitary:	(N-)	(10)	(1)	(0)	(10)
(B) Anterior adenoma		2	1	–	1
Integumentary System					
Skin:	(N-)	(10)	(1)	(0)	(10)
(B) Papilloma		0	1	–	0

Malignancy: (B) Benign neoplams (M) Malignant neoplasms
* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 58: Neoplastic lesions in male rats (main group) after terminal kill after 104 weeks of treatment

Site & Lesion	Dose (ppm)	0	40	400	4000
	No. of animals examined	36	40	43	37
Cardiovascular System					
Heart:	(N-)	(36)	(0)	(0)	(37)
(B) Schwannoma		1	–	–	0
Hematopoietic & Lymphatic System					
General:	(N-)	(36)	(40)	(43)	(37)
(M) Mononuclear cell leukemia		7	1	4	3
Thymus:	(N-)	(36)	(1)	(0)	(37)
(B) Thymoma		1	0	–	0
Respiratory System					
Lung:	(N-)	(36)	(40)	(43)	(37)
(B) Adenoma		3	4	3	5
Digestive System					
Stomach (non-glandular portion):	(N-)	(36)	(0)	(0)	(37)
(B) Papilloma		0	–	–	1
(M) Malignant schwannoma		0	–	–	1
Liver:	(N-)	(36)	(40)	(43)	(37)
(B) Hepatocellular adenoma		1	0	1	0
Pancreas:	(N-)	(36)	(0)	(1)	(37)
(B) Islet cell adenoma		2	–	0	0
Urinary System					
Kidney:	(N-)	(36)	(40)	(43)	(37)
(B) Hemangioma		0	1	0	0
Urinary bladder:	(N-)	(36)	(0)	(0)	(37)
(M) Squamous cell carcinoma		0	–	–	1
Genital System					
Ovary:	(N-)	(36)	(3)	(3)	(37)
(B) Granulosa cell tumor		0	0	0	1
Uterine horn:	(N-)	(36)	(11)	(14)	(37)
(B) Endometrial stromal polyp		9	7	12	14
(B) Adenoma		0	0	0	1
(B) Hemangioma		1	0	0	0
(B) Leiomyoma		2	0	0	0
(M) Adenocarcinoma		1	0	0	0
Uterine cervix:	(N-)	(36)	(1)	(1)	(37)
(M) Endometrial stromal sarcoma		0	0	1	0
(M) Leiomyosarcoma		0	1	0	0
Clitoral gland:	(N-)	(2)	(1)	(2)	(2)
(B) Adenoma		2	1	2	1
(M) Adenocarcinoma		0	0	0	1
Endocrine System					
Pituitary:	(N-)	(36)	(18)	(19)	(36)
(B) Anterior adenoma		13	15	18	14
(M) Anterior adenocarcinoma		1	0	0	0
Thyroid:	(N-)	(36)	(4)	(1)	(37)
(B) C-cell adenoma		3	4	1	3
Adrenal:	(N-)	(36)	(0)	(0)	(35)
(B) Pheochromocytoma		4	–	–	0
Sense Organs					
Auricle:	(N-)	(0)	(0)	(0)	(1)
(B) Schwannoma		–	–	–	1
Integumentary System					
Skin:	(N-)	(36)	(17)	(20)	(37)
(B) Keratoacanthoma		0	0	2	1
(B) Hemangioma		0	0	0	1
Mammary gland:	(N-)	(36)	(7)	(6)	(37)
(B) Adenoma		1	0	2	2
(B) Fibroadenoma		6	5	2	2

Malignancy: (B) Benign neoplams (M) Malignant neoplasms

* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 59: Neoplastic lesions in male rats (main group) of animals killed in extremis or found dead

Site & Lesion	Dose (ppm)	0	40	400	4000
	No. of animals examined	14	10	7	13
Cardiovascular System					
Heart:	(N-)	(14)	(10)	(7)	(13)
(M) Histiocytic sarcoma		0	1	0	0
Hematopoietic & Lymphatic System					
General:	(N-)	(14)	(10)	(7)	(13)
(M) Malignant lymphoma		0	1	0	0
(M) Mononuclear cell leukemia		6	3	2	7
Lymph nodes (others):	(N-)	(1)	(2)	(0)	(0)
(M) Histiocytic sarcoma		0	1	–	–
Spleen:	(N-)	(14)	(10)	(7)	(13)
(M) Histiocytic sarcoma		0	1	0	0
Respiratory System					
Lung:	(N-)	(14)	(10)	(7)	(13)
(B) Adenoma		0	0	1	0
Digestive System					
Stomach (non-glandular portion):	(N-)	(14)	(10)	(7)	(13)
(M) Squamous cell carcinoma		0	1	0	0
Pancreas:	(N-)	(14)	(10)	(7)	(13)
(B) Islet cell adenoma		0	0	1	0
Genital System					
Uterine horn:	(N-)	(14)	(10)	(7)	(13)
(B) Endometrial stromal polyp		2	2	0	1
(M) Endometrial stromal sarcoma		2	1	2	3
Uterine cervix:	(N-)	(14)	(10)	(7)	(13)
(M) Endometrial stromal sarcoma		0	1	0	0
Vagina:	(N-)	(1)	(0)	(0)	(0)
(M) Leiomyosarcoma		1	–	–	–
Clitoral gland:	(N-)	(1)	(0)	(0)	(0)
(B) Adenoma		1	–	–	–
Endocrine System					
Pituitary:	(N-)	(14)	(10)	(7)	(13)
(B) Anterior adenoma		4	5	2	4
Thyroid:	(N-)	(14)	(10)	(7)	(13)
(B) C-cell adenoma		0	1	1	1
Adrenal:	(N-)	(14)	(10)	(7)	(13)
(B) Pheochromocytoma		0	0	0	1
Nervous System					
Cerebrum:	(N-)	(14)	(10)	(7)	(13)
(M) Malignant meningioma		0	0	0	1
Sense Organs					
Harderian gland:	(N-)	(14)	(10)	(7)	(13)
(M) Malignant schwannoma		1	0	0	0
Integumentary System					
Skin:	(N-)	(14)	(10)	(7)	(13)
(B) Keratoacanthoma		0	0	1	0
(B) Fibroma		0	0	1	0
(M) Squamous cell carcinoma		1	0	0	0
(M) Hemangiosarcoma		1	0	0	0
(M) Histiocytic sarcoma		0	1	0	0
(M) Malignant fibrous histiocytoma		0	0	1	0
Integumentary System 《cont.》					
Mammary gland:	(N-)	(14)	(10)	(7)	(13)
(B) Adenoma		0	1	0	0
(B) Fibroadenoma		2	3	1	0

Malignancy: (B) Benign neoplams (M) Malignant neoplasms
* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 60: Neoplastic lesions in all female rats (main group)

Site & Lesion	Dose (ppm)	0	40	400	4000
	No. of animals examined	50	50	50	50
Cardiovascular System					
Heart:	(N-)	(50)	(10)	(7)	(50)
(B) Schwannoma		1	0	0	0
(M) Histiocytic sarcoma		0	1	0	0
Hematopoietic & Lymphatic System					
General:	(N-)	(50)	(50)	(50)	(50)
(M) Malignant lymphoma		0	1	0	0
(M) Mononuclear cell leukemia		13	4	6	10
Thymus:	(N-)	(50)	(11)	(7)	(49)
(B) Thymoma		1	0	0	0
Lymph nodes (others) :	(N-)	(1)	(2)	(0)	(0)
(M) Histiocytic sarcoma		0	1	–	–
Spleen:	(N-)	(50)	(15)	(11)	(50)
(M) Histiocytic sarcoma		0	1	0	0
Respiratory System					
Lung:	(N-)	(50)	(50)	(50)	(50)
(B) Adenoma		3	4	4	5
Digestive System					
Stomach (non-glandular portion) :	(N-)	(50)	(10)	(7)	(50)
(B) Papilloma		0	0	0	1
(M) Squamous cell carcinoma		0	1	0	0
(M) Malignant schwannoma		0	0	0	1
Liver:	(N-)	(50)	(50)	(50)	(50)
(B) Hepatocellular adenoma		1	0	1	0
Pancreas:	(N-)	(50)	(10)	(8)	(50)
(B) Islet cell adenoma		2	0	1	0
Urinary System					
Kidney:	(N-)	(50)	(50)	(50)	(50)
(B) Hemangioma		0	1	0	0
Urinary bladder:	(N-)	(50)	(10)	(7)	(50)
(M) Squamous cell carcinoma		0	0	0	1
Genital System					
Ovary:	(N-)	(50)	(13)	(10)	(50)
(B) Granulosa cell tumor		0	0	0	1
Uterine horn:	(N-)	(50)	(21)	(21)	(50)
(B) Endometrial stromal polyp		11	9	12	15
(B) Adenoma		0	0	0	1
(B) Hemangioma		1	0	0	0
(B) Leiomyoma		2	0	0	0
(M) Adenocarcinoma		1	0	0	0
(M) Endometrial stromal sarcoma		2	1	2	3
Uterine cervix:	(N-)	(50)	(11)	(8)	(50)
(M) Endometrial stromal sarcoma		0	1	1	0
(M) Leiomyosarcoma		0	1	0	0
Vagina:	(N-)	(2)	(0)	(0)	(0)
(M) Leiomyosarcoma		1	–	–	–
Clitoral gland:	(N-)	(3)	(1)	(2)	(2)
(B) Adenoma		3	1	2	1
(M) Adenocarcinoma		0	0	0	1
Endocrine System					
Pituitary:	(N-)	(50)	(28)	(26)	(49)
(B) Anterior adenoma		17	20	20	18
(M) Anterior adenocarcinoma		1	0	0	0
Thyroid:	(N-)	(50)	(14)	(8)	(50)
(B) C-cell adenoma		3	5	2	4
Adrenal:	(N-)	(50)	(10)	(7)	(48)
(B) Pheochromocytoma		4	0	0	1
Nervous System					
Cerebrum:	(N-)	(50)	(10)	(7)	(50)
(M) Malignant meningioma		0	0	0	1
Sense Organs					
Harderian gland:	(N-)	(50)	(10)	(7)	(50)
(M) Malignant schwannoma		1	0	0	0
Auricle:	(N-)	(0)	(0)	(0)	(1)
(B) Schwannoma		–	–	–	1
Integumentary System					
Skin:	(N-)	(50)	(27)	(27)	(50)
(B) Keratoacanthoma		0	0	3	1
(B) Fibroma		0	0	1	0
(B) Hemangioma		0	0	0	1
(M) Squamous cell carcinoma		1	0	0	0
(M) Hemangiosarcoma		1	0	0	0
(M) Histiocytic sarcoma		0	1	0	0
(M) Malignant fibrous histiocytoma		0	0	1	0
Mammary gland:	(N-)	(50)	(17)	(13)	(50)
(B) Adenoma		1	1	2	2
(B) Fibroadenoma		8	8	3	2*

Malignancy: (B) Benign neoplams (M) Malignant neoplasms
* and ** significantly different from control at 5% and 1% levels of probability respectively.

Table 61: Incidence of Interstitial Cell Tumour in Testis Of Male Rats

Dose (ppm)		**0**	**40**	**400**	**2000**
Killed *in extremis* Or found dead	No. of animal examined	6	14	11	49
	Incidence of tumour	1	10	8	46
Terminal kill after 104 weeks of treatment	No. of animal examined	44	36	39	0
	Incidence of tumour	42	36	36	-
All animals	No. of animal examined	50	50	50	50
	Incidence of tumour	43	46	44	46

Table 62: Tumour incidence in control male Fischer (F344/DuCrj) rats in IET studies conducted in same study conditions with SL-160 technical (termination: 1993-1995)

Organ/Tumor				Study ID/Year[a]/No. of animals examined								
				1	**2**	**3**	**4**	**5**	**6**	**7***	**8**	**9**
		total	%	1993	1993	1994	1993	1994	1995	1995	1995	1995
		449		50	50	49	50	50	50	50	50	50
Systemic tumor	Malignant lymphoma	1	0.22	0	0	0	0	0	0	0	1	0
	Histiocytic sarcoma	2	0.45	0	0	0	0	0	2	0	0	0
	Mononuclear cell leukemia	66	14.70	11	7	11	5	5	6	5	4	12
Skin/subcutaneous tissue	Squamous cell papilloma	1	0.22	0	0	0	0	0	0	0	0	1
	Squamous cell carcinoma	8	1.78	0	0	1	0	0	5	2	0	0
	Keratoacanthoma	24	5.35	2	4	4	2	1	1	3	6	1
	Trichoepithelioma	1	0.22	0	0	0	0	0	0	0	1	0
	Sebaceous gland adenoma	2	0.45	0	0	0	2	0	0	0	0	0
	Basal cell adenoma	1	0.22	0	0	0	1	0	0	0	0	0
	Basal cell carcinoma	2	0.45	0	0	0	1	0	1	0	0	0
	Fibroma	43	9.58	6	5	6	3	4	5	4	4	6
	Lipoma	8	1.78	1	0	0	1	0	3	2	1	0
	Liposarcoma	1	0.22	0	0	1	0	0	0	0	0	0
	Hemangiosarcoma	3	0.67	0	1	0	1	1	0	0	0	0
	Malignant schwannoma	3	0.67	0	1	1	0	0	1	0	0	0
	Malignant fibrous histiocytoma	1	0.22	0	0	0	0	0	0	0	1	0
	Histiocytic sarcoma	2	0.45	0	1	0	1	0	0	0	0	0
	Papilloma	12	2.67	0	0	3	0	1	0	3	4	1
Mammary gland	Fibroadenoma	8	1.78	0	2	1	0	1	0	2	1	1
	Fibroma	2	0.45	0	0	0	0	2	0	0	0	0
	Adenocarcinoma	1	0.22	0	0	0	0	0	0	0	1	0
Spleen	Hemangioma	1	0.22	0	1	0	0	0	0	0	0	0
	Hemangiosarcoma	2	0.45	0	0	0	0	0	1	1	0	0
	Histiocytic sarcoma	1	0.22	0	0	0	0	0	0	1	0	0

Thymus	Malignant thymoma	1	0.22	1	0	0	0	0	0	0	0	0
Bone	Histiocytic sarcoma	1	0.22	0	0	0	1	0	0	0	0	0
	Osteosarcoma	1	0.22	1	0	0	0	0	0	0	0	0
Lung	Adenoma	39	8.69	2	5	5	6	6	3	5	4	3
	Adenocarcinoma	3	0.67	2	0	0	0	0	0	0	1	0
Heart	Schwannoma	11	2.45	1	0	1	1	3	0	3	2	0
	Malignant schwannoma	1	0.22	0	0	0	0	0	0	1	0	0
Oral cavity	Squamous cell carcinoma	1	0.22	0	0	1	0	0	0	0	0	0
Forestomach	Squamous cell carcinoma	1	0.22	0	0	0	0	0	0	0	1	0
	Papilloma	1	0.22	1	0	0	0	0	0	0	0	0
Glandular stomach	Leiomyosarcoma	1	0.22	1	0	0	0	0	0	0	0	0
Small intestine	Adenoma	2	0.45	0	0	0	0	0	0	1	1	0
	Adenocarcinoma	1	0.22	0	0	0	0	0	0	0	1	0
	Leiomyosarcoma	1	0.22	0	0	1	0	0	0	0	0	0
Liver	Hepatocellular adenoma	18	4.01	2	0	1	4	1	1	6	2	1
	Histiocytic sarcoma	1	0.22	0	0	1	0	0	0	0	0	0
Pancreas	Islet cell adenoma	27	6.01	3	6	2	2	3	1	7	2	1
	Islet cell carcinoma	1	0.22	0	0	0	0	0	0	0	1	0
	Acinar cell adenoma	2	0.45	0	0	0	2	0	0	0	0	0
Kidney	Adenoma	4	0.89	0	0	0	3	0	0	0	1	0
	Transitional cell carcinoma	1	0.22	0	0	0	0	0	0	0	0	1
	Nephroblastoma	1	0.22	0	0	1	0	0	0	0	0	0
Urinary bladder	Transitional cell papilloma	3	0.67	0	1	0	2	0	0	0	0	0
Testis	Interstitial cell tumor	400	89.09	47	44	37	42	43	48	43	50	46
Prostate	Adenoma	1	0.22	0	0	0	0	1	0	0	0	0
Preputial gland	Adenoma	11	2.45	2	0	0	2	0	1	3	1	2
	Carcinoma	3	0.67	0	1	1	0	0	0	1	0	0
Pituitary	Anterior adenoma	107	23.83	9	8	19	10	12	12	7	15	15
	Adenoma in intermediate pari	1	0.22	0	0	0	0	0	0	1	0	0
Thyroid	C-cell adenoma	101	22.49	14	12	15	6	8	9	14	10	13
	Papillary adenoma	1	0.22	0	0	1	0	0	0	0	0	0
	C-cell carcinoma	2	0.45	0	0	0	1	0	1	0	0	0
	Follicular cell adenoma	4	0.89	0	0	1	0	0	2	0	1	0
	Follicular cell carcinoma	1	0.22	0	0	1	0	0	0	0	0	0
Parathyroid	Adenoma	1	0.22	0	0	0	0	0	0	0	0	1

Adrenal	Cortical adenoma	1	0.22	0	0	0	0	0	0	1	0	0
	Pheochromocytoma	68	15.14	8	6	10	9	8	10	10	3	4
	Malignant pheochromocytoma	5	1.11	1	1	0	1	0	0	0	2	0
	Ganglioneuroma	2	0.45	0	0	0	0	0	0	0	2	0
Brain	Glioma	2	0.45	0	0	1	0	0	0	0	0	1
	Malignant glioma	3	0.67	0	1	0	0	0	2	0	0	0
	Granular cell tumor	1	0.22	0	0	0	0	0	0	1	0	0
Spinal cord	Glioma	1	0.22	0	0	1	0	0	0	0	0	0
Auricle	Melanocytic tumor	1	0.22	0	0	1	0	0	0	0	0	0
Ear	Zymbal's gland carcinoma	3	0.67	0	0	2	0	1	0	0	0	0
Zymbal's gland	Carcinoma	1	0.22	0	0	0	0	0	1	0	0	0
Thoracic cavity	Malignant mesothelioma	1	0.22	0	0	0	0	0	1	0	0	0
	Neurofibrosarcoma	1	0.22	0	0	1	0	0	0	0	0	0
	Malignant schwannoma	1	0.22	0	0	0	0	0	0	0	1	0
Abdominal cavity	Malignant mesothelioma	15	3.34	2	4	3	1	1	1	2	0	1
	Malignant schwannoma	1	0.22	0	0	0	0	0	0	0	0	1
	Liposarcoma	1	0.22	0	0	0	0	1	0	0	0	0
	Fibrosarcoma	1	0.22	0	0	0	0	0	0	1	0	0
	Cordoma	1	0.22	0	0	1	0	0	0	0	0	0
	Malignant fibrous histiocytoma	1	0.22	0	0	0	0	0	1	0	0	0

[a]: Year at termination of treatment
The above data were obtained from untreated control animals in carcinogenicity studies conducted in the Institute of Environmental Toxicology (IET) during 5 years
*: SL-160 technical study

Since no evidence of carcinogenicity was observed at tested dose levels, the **NOAEL for carcinogenicity** was considered to be greater than **2000/4000 ppm, equivalent to >70.1/ 172.6 mg/kg bw/day for males/females**. **NOAEL for toxicity** was considered **40 ppm,** corresponding to **1.313 and 1.601 mg/kg bw/day for males and females**, respectively.

In an 18-month oncogenicity study in mice (Anonymous 21, 1995a; B.6.5.2), flazasulfuron was tested at dose levels of 0, 500, 3500 and 7000 ppm, equivalent to 0, 70.4, 497.8 and 987.4 mg/kg bw/day for males and 0, 88.5, 596.4 and 1165.5 mg/kg bw/day for females.

The rationale for selection of dose levels in this study arises from a previous 6-week feeding study in mice (Anonymous 38, 1992; B.6.3.1.1). In this study, groups of 10 mice per each sex were fed flazasulfuron at 0, 200, 1000, 5000 and 10000 ppm dose levels over a 6-week period. Most importantly, terminal bodyweight was statistically significantly lower for both sexes in the high dose group, and a treatment-related effects were observed in the necropsy and histopathological examinations of the livers in the 5000 and 10000 ppm dose groups. Absolute and relative liver weights were statistically significantly higher in males of the 5000 and 10000 ppm dose groups, and in females of high dose group. In addition, histopathologic analysis revealed centrilobular hepatocyte hypertrophy in both sexes at 5000 and 10000 dose levels. Thus, the definitive doses of 0, 500, 3500 and 7000 ppm were selected for the main oncogenicity study.

In the present study, a slight increase in mortality was observed in all dose males groups from week 52-65 (6 deaths for each dose group vs 1 death at control group), although at termination of study at week 80, the cumulative deaths were more evenly distributed between all males groups (8, 10, 15 and 10 for 0, 500, 3500 and 7000 ppm dose groups, respectively). Similar result was observed in females groups at termination of study, although no differences were observed in previous weeks. Data displayed no statistical differences

between different groups for both sexes; however, MSCA considers that an effect in mortality related to treatment in male dose groups previous termination of study should have been evaluated by authors.

For males at high dose group, bodyweights tended to be lower than in the control animals throughout the study, together with bodyweights gains until the week 12. In the mid dose male group, lower bodyweights values were measured until week 25, however, no differences were observed in bodyweight gains at any week. All male study groups had similar bodyweights at randomization, but the 7000 ppm dose group had statistically significantly lower mean bodyweight at week 0 (4.1%) prior to test material administration. These deviations at week 0, prior test substance feed exposure, explain the decreased bodyweights observed in the high dose males throughout the study period. Besides, these observations were not associated with depression in food consumption, so it is unlikely that these changes were treatment-related.

On the other hand, females showed statistical1y significant lower mean bodyweights and bodyweight gains throughout the study in the mid and high dose groups compared with the control group. The observation of dose-relationship determines that these differences were considered treatment related.

Food consumption, both absolute and relative to bodyweight, displayed both increases and decreases measurements during treatment period in all dose male groups. These observations were not considered treatment-related due to there has no trend over the study. Absolute and relative food consumption was statistically significantly lower in mid and high dose female groups throughout the treatment period, and these differences were attributed to test substance administration.

Haematology analysis only included leukocyte count, so MSCA deems that measurements of other blood parameters had to be included. Eosinophils were statistically significantly higher at 12 and 18 months for the high dose males (135% and 73%, respectively) and females (68% and 76%, respectively), when compared to the controls. These values were associated to treatment, but the toxicological significance is undetermined because of many factors could be involved. Statistically significant deviations observed in monocyte or lymphocyte counts in all treated groups for males and females were not dose-related, and consequently were not attributable to test substance administration.

Ophthalmological examinations, clinical biochemistry and urinalysis were not performed in the study.

The observations at necropsy were of the type expected in a long-term mouse study and there was no indication of a treatment-related effect on gross pathology for both sexes.

Regarding organ weights, males and females at mid and high dose groups exhibited a statistically significant increase of absolute and relative liver weights. An increase of absolute and relative liver was observed in male (24% and 23%, respectively) and female (24% and 33%, respectively) high dose groups. At mid dose groups, an increase of absolute and relative liver (22% and 21%, respectively) was observed in males, whereas an increase of 19% on relative liver weight was noted in females. This result is correlated with higher incidences of histopathological defects in liver of mid and high dose groups for both sexes.

Histopathological evaluation showed an increase of hepatocellular hypertrophy incidences for both sexes in the mid and high dose groups, that probably accounted for the increase in liver weight observed in these dose groups. In addition, a slightly increased incidence of hepatocellular pigment was detected in the high dose male group. Both effects were considered treatment-related. Furthermore, there were no evidences of a substance-related increase in other non-neoplastic findings.

There were no evidence of a treatment related increases in neoplasms. The only comparison of neoplastic findings between groups included in the original study report corresponded to hepatocellular adenomas and/or carcinomas for which Mantel-Haenszel statistic was employed for a dose related trend and Fisher’s exact test to compare treated and control groups. The incidence of hepatocellular adenoma were analysed separately for each sex. Then the incidences of hepatocellular adenoma and carcinoma were combined and analysed. Statistical analysis indicated no significant dose related trend and no significant difference between gropus.

Table 63: Summary of the frequency of relevant neoplastic findings

Sex	Neoplastic findings	Dose (ppm)			
		0	500	3500	7000
Male	Hepatocellular adenoma*	5	7	10	7
	Hepatocellular carcinoma	1	1	0	2
	Total	6	8	10	8
Female	Hepatocellular adenoma*	1	0	3	1
	Hepatocellular carcinoma	0	0	0	0
	Total	1	0	3	1

*including both sinble and multiple hepatocellular adenoma

Since no evidence of carcinogenicity was observed at tested dose levels, the **NOAEL for carcinogenicity** was considered to be greater than **7000 ppm, equivalent to >987.4 and 1165.5 mg/kg bw/day for males and females, respectively**. **NOAEL for toxicity** was considered **500 ppm,** corresponding to **70.4 and 88.5 mg/kg bw/day for males and females**, respectively.

Table 64: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Not applicable due to the lack of evidence for carcinogenicity								

10.9.2 Comparison with the CLP criteria

According to CLP criteria (Regulation (EC) Nº 1272/2008), a carcinogen is a substance or a mixture that induces cancer or increases its incidence. Substances that have induced benign and malignant tumours in well-performed experimental studies on animals are also considered to be presumed or suspected human carcinogens, unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.

Carcinogenic substances are allocated to Category 1 (known or presumed human carcinogens) or Category 2 (suspected human carcinogens).

Substances are classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. Substances are classified in Category 1A when known to have carcinogenic potential for humans (based largely on human evidence). Substances are classified in Category 1B when presumed to have carcinogenic potential for humans (based largely on animal evidence). The classification in Category 1A and 1B is based on strength of evidence. Human studies should establish a causal relationship between human exposure to a substance and the development (known human carcinogen). On the other hand, animal experiments must show a sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

Based on the data available, there was no evidence of flazasulfuron induced cancer or increased its incidence. Two chronic toxicity/carcinogenicity studies were submitted and assessed, in which toxicity of test substance was investigated in two different species (rat andmice). No increased rate of malignant neoplasms, or a

combination of benign and malignant neoplasms, was detected in the studies. Consequently, according to criteria of the Regulation (EC) No. 1272/2008 no classification for carcinogenicity is warranted.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the data available, and according to the criteria under Regulation (EC) No. 1272/2008, flazasulfuron does not meet the classification criteria for carcinogenicity. The data is considered **conclusive but not sufficient for classification**.

10.10 Reproductive toxicity

10.10.1Adverse effects on sexual function and fertility

Table 65: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
Range-finding study: **A single generation reproduction study in rats** Method: No guideline GLP: Yes Rat strain: Sprague-Dawley (CD® –Crl : (SD) BR) Sex: ♂ and ♀ No. animals: P: 16 rats/sex/dose. Deviations from current test guideline (OECD TG 416, 2001): -16 pairs of animals were used per group, instead of 20 animals at or near parturition. -The dose interval exceed the 3 fold recommended for dietary studies.	Test substance: **Flazasulfuron [**SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.1% purity. Oral: Diet 0, 100, 500, 2500 and 10000 ppm equivalent to: ♂: 0, 7, 34, 175 and 731 mg/kg bw/day*. ♀: 0, 8, 40, 196 and 791 mg/kg bw/day*. Exposure: *Pre-mating treatment:* P: 77 days (10 weeks) *Mating:* 15 days Treatment continued in P and F_1 throughout gestation and lactation. Parameters observed *Parental toxicity:* Mortality, clinical signs, bw, bw gain and food consumption. *Development (F_1 offspring):* Mean litter size, pup sex ratios, number of live and stillborn pups	**PARENTAL TOXICITY (P)** Mortality: no mortality or morbidity signs were observed in animals at any dose level. One male rat from 2500 ppm dose group was found dead in week 2 of the study. Clinical signs: No treatment related clinical signs were observed. **10000 ppm** (731 ♂/ 791 ♀ mg/kg bw/day) *Bodyweight and bodyweight gain* *Premating* ▪ (↓) bw in ♂ throughout week 1- 10 (16-17%). ▪ (↓) bwg in ♂ throughout week 0- 10 (25%). ▪ (↓) bw in ♀ throughout week 1- 10 (10-12%). ▪ (↓) bwg in ♀ throughout week 0- 10 (27%). *Gestation* ▪ (↓) bw in ♀ at gestation days 0 (15%), 7 (15%), 14 (13%) and 20 (14%). *Lactation* ▪ (↓) bw in ♀ at lactation days 0 (14%), 7 (11%), 14 (8%) and 21 (7%). *Food consumption* *Premating* ▪ (↓) abs (g/day) in ♂ throughout week 1- 9 (14-16%). ▪ (↑) rel to bw (g/kg bw/day) in ♂ at week 3 (7%, ndr), 4 (8%, ndr), 5 (10%, ndr), 6 (7%, ndr) and 10 (10%, ndr). ▪ (↓) abs in ♀ throughout week 3- 9 (9-15%). **2500 ppm** (175 ♂/ 196 ♀ mg/kg bw/day) *Bodyweight and bodyweight gain* *Premating* ▪ (↓) bw in ♂ throughout week 1- 8 (7-10%). ▪ (↓) bwg in ♂ throughout week 0- 10 (14%). ▪ (↓) bwg in ♀ throughout week 0- 10 (15%). *Gestation* ▪ (↓) bw in ♀ at gestation days 7 (8%), 14 (8%) and 20 (7%). **500 ppm** (34 ♂/ 40 ♀ mg/kg bw/day) *Bodyweight*	Anonymous 23 (1993) (CA) B.6.6.1.1

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>-Food consumption not measured in reproduction phases.

-No. of implantations, corpora lutea and post-implantation loss were not measured.

-Organ weights were not recorded (uterus, ovaries, testes, epididymides, prostate, seminal vesicles, brain, liver, kidney, etc.)

Histopathological examination in sexual organs was not performed.

-Sperm parameters were not measured.

-Macroscopic examination and gross necropsy were not conducted.

Acceptable as preliminary study</td>
<td>at birth, day 4 pup viability index, lactation index, pup wt, external abnormalities.

<u>Sexual function and fertility</u>:
Mating idex: no. of ♀ with postive signs of mating / total no. of ♀

Fertility index: no. of pregnant ♀/ no. of ♀ with postive signs of mating

Sex ratio: total no. males/total live born pups

Gestation length

*Equivalences are based on mean compound consumption throughout premating period (10 weeks). Equivalences in RAR 2016 are only based on week 10.</td>
<td><u>Lactation</u>
▪ (↑) bw in ♀ at lactation day 0 (7%, ndr).

<u>SEXUAL FUNCTION AND FERTILITY</u>

Summary of reproductive indices
<table>
<tr><th>Dose (ppm)</th><th>Mating Index</th><th>Fertility Index</th><th>Gestation lenght (d)</th><th>Sex ratio (males)</th></tr>
<tr><td>0</td><td>16/16 (100%)</td><td>16/16 (100%)</td><td>22.3</td><td>49.2</td></tr>
<tr><td>100</td><td>13/16 (81%)</td><td>12/13 (92%)</td><td>22.3</td><td>43.9</td></tr>
<tr><td>500</td><td>15/16 (94%)</td><td>14/15 (93%)</td><td>22.4</td><td>58.0</td></tr>
<tr><td>2500</td><td>15/15 (100%)</td><td>15/15 (100%)</td><td>22.3</td><td>54.7</td></tr>
<tr><td>10000</td><td>13/16 (81%)</td><td>12/13 (92%)</td><td>22.5</td><td>48.4</td></tr>
</table>
The reduction in the number of live fetuses (F1) could be associated to impairment of sexual function or fertility and also for development since the no. of implantations was not measured in this study. Consequently it is included in both sections. In any case, the effect was regarded incidental (within HCD range and not significant at the highest dose level of 10000 ppm).

<u>DEVELOPMENTAL TOXICITY (F_1)</u>
It has to be noted that the effects on live born pups could be associated to developmental or fertility impairment since the no. of implantations was not measured.

<u>10000 ppm</u> (731 ♂/ 791 ♀ mg/kg bw/day)
▪ (↓) live born pups (19% but not statistically significant).
▪ (↓) total pups (19%, statistically significant).
▪ (↓) bw at day 14 (7%) and 21 (10%).

Mean live born pups per litter
<table>
<tr><th>HCD (1987-1991) study</th><th>year</th><th>Range</th></tr>
<tr><td>1722-87-0121-TX-003</td><td>1987</td><td rowspan="3">11.3-14.5</td></tr>
<tr><td>1703-88-0176-TX-003</td><td>1988</td></tr>
<tr><td>3822-91-0014-TX-003</td><td>1991</td></tr>
<tr><th>Dose (ppm)</th><th>year</th><th>mean live born pups (no.litters)</th></tr>
<tr><td>0</td><td rowspan="5">1993</td><td>16.5 (16)</td></tr>
<tr><td>100</td><td>15.4 (12)</td></tr>
<tr><td>500</td><td>14.2 (14)</td></tr>
<tr><td>2500</td><td>13.8* (15)</td></tr>
<tr><td>10000</td><td>13.3 (12)</td></tr>
<tr><th>HCD (1992-1996) study</th><th>year</th><th>mean live born pups (no.litters)</th><th>Range</th></tr>
<tr><td>5330-92-0223-TX-004</td><td>1992</td><td>F1= 14.1 F2=12.7</td><td rowspan="3">12.7-14.1</td></tr>
<tr><td>6755-96-0042-TX-003</td><td>1992</td><td>F1= 13.6 F2=13</td></tr>
<tr><td>6754-96-0041-TX-001</td><td>1996</td><td>13.6</td></tr>
</table>
*p<0.05 from control group in the same study.

<u>500 ppm</u> (34 ♂/ 40 ♀ mg/kg bw/day)</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		▪ (↑) bw at day 21 (8%, ndr). **NOAEL** developmental toxicity**:** set in RAR (2016) at **2500 ppm** (175 and 196 mg/kg bw/day for males and females) based on the decreased pups weight noted on lactation days 14 and 21 at 10000 ppm dose group. However, this decrease is regarded in this CLH Report of doubtful toxicological relevance due to the presence of maternal toxicity as it is further discussed in section 10.10.6. **NOAEL** sexual function and fertility**: 10000 ppm** (731 and 791 mg/kg bw/day for males and females) since no effects on sexual function and fertility were detected at any dose level. **NOAEL** parental toxicity**: 500 ppm** (34 and 40 mg/kg bw/day for males and females) based on the decreased bodyweight and bodyweight gains observed at 2500 ppm dose groups in both sexes.	
A two-generation reproduction study in rats Method: US EPA 83-4 comparable to OECD TG 416 (1983) GLP: Yes Rat strain: Sprague-Dawley (CD® –Crl : (SD) BR) Sex: ♂ and ♀ No. animals: P: 30 rats/sex/dose. Deviations from current test guideline (OECD TG 416, 2001): -The dose interval exceed the 3 fold recommended for dietary studies. -Food consumption not measured during reproductive phases. -No. of	Test substance: **Flazasulfuron [**SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.1% purity. Oral: Diet 0, 200, 2000, and 10000 ppm equivalent to: ♂: 0, 14, 141, and 717 mg/kg bw/day*. ♀: 0, 16, 160, and 801 mg/kg bw/day*. Exposure: *Pre-mating treatment:* P: 77 days (10 weeks) F_1: 84 days (12 weeks) *P and F_1 mating :* 15 days Treatment continued in P, F_1 and F_2 throughout gestation and lactation. Parameters observed *P/F_1 parental animals:* Mortality, clinical signs, bw, bw gain and food consumption. *F_1/F_2 offspring:* Mean litter size, pup sex ratios, number of live and stillborn pups	**PARENTAL TOXICITY (P and F_1)** Mortality: no gross mortality or morbidity signs were associated to test substance administration. ▪ 1 P male control group was killed in moribund conditions at week 16. ▪ 2 F_1 parental animals died during the study: 1 female at 2000 ppm dose group died at week 16 due to hyperplasia of the ureter and urinary bladder associated with urolithiasis, but these lesions were not considered to be compound related. 1 female at 10000 ppm dose group died at week 20 with severe effects in the kidneys that may have resulted in death. ▪ 1 F_1 female of the 2000 ppm dose group was sacrificed at week 24 due to moribund conditions. The necropsy examination revealed that had lymphosarcoma in the lymph nodes, spleen and numerous parenchyma organs but not considered to be treatment related. Clinical signs: No treatment related clinical signs were observed. **10000 ppm** (717 ♂/801 ♀ mg/kg bw/day) ***P*** *Bodyweight and bodyweight gain* *Premating* ▪ (↓) bw in ♂ throughout week 1- 10 (14-17%). ▪ (↓) bwg in ♂ throughout week 0- 10 (30%). ▪ (↓) bw in ♀ throughout week 1- 10 (9-13%). ▪ (↓) bwg in ♀ throughout week 0- 10 (29%). *Gestation* ▪ (↓) bw in ♀ at gestation days 0 (15%), 7 (15%), 14 (15%) and 20 (13%). *Lactation* ▪ (↓) bw in ♀ at lactation days 0 (15%), 7 (15%), 14 (15%) and 21 (12%). *Food consumption* ▪ (↓) abs (g/day) in ♂ throughout week 1- 10 (14-43%). ▪ (↓) rel to bw (g/kg bw/day) in ♂ at week 1 (34%) and (↑) throughout week 2-10 (3-5%, ncdr). ▪ (↓) abs (g/day) in ♀ throughout week 1-10 (7-35%). ▪ (↓) rel to bw (g/kg/bw/day) in ♀ at week 1 (28%) and week 2 (4%). (↑) at week 10 (7%, ndr). *Efficiency of food consumption*	Anonymous 24 (1995b) (CA) B.6.6.1.2

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>implantations, corpora lutea and post-implantation loss were not analysed.

-Organ weights were not recorded (uterus, ovaries, testes, epididymides, prostate, seminal vesicles, brain, liver, kidney, etc.)

-Sperm parameters were not measured.

-Quantitative evaluation of primordial follicles was not performed.

Acceptable study</td>
<td>at birth, day 4 pup viability index, lactation index, pup wt, external abnormalities, and histopathological analysis.

<u>Reproductive</u>:
Mating idex: no. of ♀ with postive signs of mating / total no. of ♀

Fertility index: no. of pregnant ♀/ no. of ♀ with postive signs of mating

Sex ratio: total no. males/total live born pups

Gestation length

*Equivalences are based on mean compound consumption throughout F_1 and F_2 premating period (10 weeks in F_1 and 12 weeks in F_2 generations). Equivalences in RAR 2016 are only based on week 10 of F_1 generation.</td>
<td>▪ (↓) in ♂ at week 1 (58%) and 8 (29%).
▪ (↓) in ♀ at week 1 (62%), 2 (24%) and (↑) at week 6 (53%, ndr).

<u>Necropsy findings (only ♂)</u>
Kidney
▪ (↑) bilateral discoloration (27%; 8/30 animals vs 0/30 in controls).
▪ (↑) both enlarged (10%; 3/30 animals vs 1/30 in controls).
▪ (↑) both pale (27%; 8/30 animals vs 0/30 in controls).
▪ (↑) dilated pelvis (13%; 4/30 animals vs 0/30 in controls).

Necropsy findings (P)
<table>
<tr><th colspan="5">Incidence in group</th></tr>
<tr><th>Dose level (ppm)</th><th>0</th><th>200</th><th>2000</th><th>10000</th></tr>
<tr><td>No. of animals examined</td><td>30</td><td>30</td><td>30</td><td>30</td></tr>
<tr><td colspan="5">Kidney findings</td></tr>
<tr><td>Bilateral discoloration</td><td>0</td><td>1</td><td>3</td><td>8</td></tr>
<tr><td>Both enlarged</td><td>1</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Right granular</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Both mottled</td><td>1</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>Both pale</td><td>0</td><td>1</td><td>3</td><td>8</td></tr>
<tr><td>Dilated pelvis</td><td>0</td><td>0</td><td>3</td><td>4</td></tr>
</table>

<u>Histopathology (only ♂)</u>
Kidney
▪ (↑) slight/mild nephropathy (57%; 17/30 animals vs 4/30 in controls).
▪ (↑) moderate nephropathy (23%; 7/30 animals vs 0/30 in controls).
▪ (↑) moderately severe nephropathy (3%; 1/30 animals vs 0/30 in controls).
▪ (↑) minimal tubular dilation (63%; 19/30 animals vs 0/30 in controls).
▪ (↑) slight/mild tubular dilation (30%; 9/30 animals vs 0/30 in controls).

Histopathological kidney finding in P animals
<table>
<tr><th colspan="5">Incidence in group</th></tr>
<tr><th>Dose level (ppm)</th><th>0</th><th>200</th><th>2000</th><th>10000</th></tr>
<tr><td>No. of animals examined</td><td>30</td><td>30</td><td>30</td><td>30</td></tr>
<tr><td colspan="5">Kidney findings</td></tr>
<tr><td><u>Nephropathy</u></td><td>18</td><td>25</td><td>28</td><td>30</td></tr>
<tr><td>Minimal</td><td>14</td><td>17</td><td>22</td><td>5</td></tr>
<tr><td>Slight/mild</td><td>4</td><td>7</td><td>2</td><td>17</td></tr>
<tr><td>Moderate</td><td>0</td><td>1</td><td>3</td><td>7</td></tr>
<tr><td>Moderately severe</td><td>0</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td><u>Tubular dilation</u></td><td>0</td><td>0</td><td>22</td><td>28</td></tr>
<tr><td>Minimal</td><td>0</td><td>0</td><td>21</td><td>19</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

Slight/mild	0	0	1	9	

F₁ adults

Bodyweight and bodyweight gain

Premating

- (↓) bw in ♂ throughout week 1- 12 (21-24%).
- (↓) bwg in ♂ throughout week 0- 12 (23%).
- (↓) bw in ♀ throughout week 1- 12 (14-17%).
- (↓) bwg in ♀ throughout week 0- 12 (14%).

Gestation

- (↓) bw in ♀ at gestation days 0 (15%), 7 (16%), 14 (17%) and 20 (15%).

Lactation

- (↓) bw in ♀ at lactation days 0 (17%), 7 (16%), 14 (14%) and 21 (8%).

Food consumption

- (↓) abs (g/day) in ♂ throughout week 1- 12 (19-21%).
- (↑) rel to bw (g/kg bw/day) in ♂ at week 1 (6%, ncdr), 2 (6%, ncdr), 7 (5%, ncdr) and 8 (5%, ncdr).
- (↓) abs (g/day) in ♀ throughout week 1-12 (12-17%).

Efficiency of food consumption

- (↓) in ♂ at week 1 (9%), 2 (4%) and 6 (10%). (↑) at week 5 (10%, ndr).
- (↑) in ♀ at week 2 (11%, ndr).

Necropsy findings (only ♂)

Kidney

- (↑) bilateral discoloration (53%; 16/30 animals vs 0/30 in controls).
- (↑) both enlarged (87%; 26/30 animals vs 0/30 in controls).
- (↑) both granular (43%; 13/30 animals vs 0/30 in controls).
- (↑) both pale (10%; 3/30 animals vs 0/30 in controls).
- (↑) dilated pelvis (50%; 15/30 animals vs 5/30 in controls).

Necropsy findings (F_1)

Incidence in group				
Dose level (ppm)	**0**	**200**	**2000**	**10000**
No. of animal examined	30	30	30	30
Kidney findings				
Bilateral discoloration	0	0	4	16
Both enlarged	0	0	5	26
Right granular	0	0	0	13
Both mottled	1	0	1	2
Both pale	0	0	1	3
Dilated pelvis	5	4	7	15

Histopathology

Kidney

- (↑) slight/mild nephropathy in ♀ (30%; 9/30 animals vs 0/30 in controls).

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
▪ (↑) moderate nephropathy in ♂ (30%; 9/30 animals vs 1/30 in controls) and ♀ (3%; 1/30 animals vs 0/30 in controls).
▪ (↑) moderate severe nephropathy in ♂ (67%; 20/30 animals vs 0/30 in controls) and ♀ (7%; 2/30 animals vs 0/30 in controls).
▪ (↑) severe/high nephropathy in ♂ (3%; 1/30 animals vs 0/30 in controls).
▪ (↑) slight/mild tubular dilation in ♀ (10%; 3/30 animals vs 0/30 in controls).
▪ (↑) moderate tubular dilation in ♂ (13%, 4/30 animals vs 0/30 in controls), and ♀ (20%; 6/30 animals vs 0/30 in controls).
▪ (↑) moderately severe tubular dilation in ♂ (47%; 14/30 animals vs 0/30 in controls) and in ♀ (50%; 15/30 animals vs 0/30 in controls).
▪ (↑) severe/high tubular dilation in ♂ (40%, 12/30 animals vs 0/30 in controls), and ♀ (20%; 6/30 animals vs 0/30 in controls).
▪ (↑) minimal cystic tubules in ♀ (20%; 6/30 animals vs 0/30 in controls)
▪ (↑) slight/mild cystic tubules in ♂ (7%; 2/30 animals vs 0/30 in controls) and in ♀ (33%; 10/30 animals vs 0/30 in controls).
▪ (↑) moderate cystic tubules in ♂ (43%; 13/30 animals vs 0/30 in controls) and in ♀ (17%; 5/30 animals vs 0/30 in controls).
▪ (↑) moderately severe cystic tubules in ♂ (17%; 5/30 animals vs 0/30 in controls).
▪ (↑) severe/high cystic tubules in ♂ (33%; 10/30 animals vs 0/30 in controls).
▪ (↑) congestion of veins in the renal medulla ♂ (47%; 14/30 animals vs 0/30 in controls).

Nephropathy incidences (F_1)
<table>
<tr><th colspan="5">Incidence in group</th></tr>
<tr><th>Dose level (ppm)</th><th>0</th><th>200</th><th>2000</th><th>10000</th></tr>
<tr><th colspan="5">Male kidney findings</th></tr>
<tr><td>No. of animals examined</td><td>30</td><td>30</td><td>30</td><td>30</td></tr>
<tr><td><u>Nephropathy</u></td><td>17</td><td>23</td><td>29</td><td>30</td></tr>
<tr><td>Minimal</td><td>11</td><td>20</td><td>3</td><td>0</td></tr>
<tr><td>Slight/mild</td><td>5</td><td>3</td><td>6</td><td>0</td></tr>
<tr><td>Moderate</td><td>1</td><td>0</td><td>18</td><td>9</td></tr>
<tr><td>Moderately severe</td><td>0</td><td>0</td><td>2</td><td>20</td></tr>
<tr><td>Severe/high</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><th colspan="5">Female kidney findings</th></tr>
<tr><td>No. of animals examined</td><td>30</td><td>30</td><td>30</td><td>30</td></tr>
<tr><td><u>Nephropathy</u></td><td>4</td><td>6</td><td>9</td><td>26</td></tr>
<tr><td>Minimal</td><td>4</td><td>6</td><td>8</td><td>14</td></tr>
<tr><td>Slight/mild</td><td>0</td><td>0</td><td>1</td><td>9</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Moderately severe</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Severe/high</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

Other kidney findings (F_1)

Incidence in group				
Dose level (ppm)	**0**	**200**	**2000**	**10000**
Males				
No. of animal examined	30	30	30	30
Tubular dilation	0	1	0	0
Minimal	0	1	0	0
Slight/mild	0	0	2	0
Moderate	0	0	15	4
Moderately severe	0	0	11	14
Severe/high	0	0	2	12
Cystic tubules	0	0	11	30
Minimal	0	0	1	0
Slight/mild	0	0	6	2
Moderate	0	0	4	13
Moderately severe	0	0	0	5
Severe/high	0	0	0	10
Females				
No. of animals examined	30	30	30	30
Tubular dilation	0	1	4	30
Minimal	0	1	3	0
Slight/mild	0	0	1	3
Moderate	0	0	0	6
Moderately severe	0	0	0	15
Severe/high	0	0	0	6
Cystic tubules	0	0	0	21
Minimal	0	0	0	6
Slight/mild	0	0	0	10
Moderate	0	0	0	5
Moderately severe	0	0	0	0
Severe/high	0	0	0	0

2000 ppm (141 ♂/160 ♀ mg/kg bw/day)

P

Bodyweight and bodyweight gain
Premating

- (↓) bw in ♂ throughout week 1- 10 (5-6%).
- (↓) bwg in ♂ throughout week 0- 10 (9%).
- (↓) bwg in ♀ throughout week 0- 10 (8%)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		Lactation ▪ (↓) bw in ♀ at lactation days 7 (5%), 14 (5%) and 21 (6%). *Food consumption* ▪ (↓) abs (g/day) in ♂ through week 1- 4 (8-9%) and at week 9 (7%). *Efficiency of food consumption* ▪ (↓) in ♂ at week 1 (15%) and 8 (25%). ▪ (↓) in ♀ at week 1 (19%). *Necropsy findings (only ♂)* *Kidney* ▪ (↑) bilateral discoloration (10%; 3/30 animals vs 0/30 in controls). ▪ (↑) both pale (10%; 3/30 animals vs 0/30 in controls). ▪ (↑) dilated pelvis (10%; 3/30 animals vs 0/30 in controls). *Histopathology (only ♂)* *Kidney* ▪ (↑) moderate nephropathy (10%; 3/30 animals vs 0/30 in controls). ▪ (↑) moderately severe nephropathy (3%; 1/30 animals vs 0/30 in controls). ▪ (↑) minimal tubular dilation (70%; 21/30 animals vs 0/30 in controls). ▪ (↑) slight/mild tubular dilation (3%; 1/30 animals vs 0/30 in controls). ***F_1 adults*** *Bodyweight and bodyweight gain* *Premating* ▪ (↓) bw in ♂ through week 1- 6 (5-6%). ▪ (↓) bwg in ♂ at week 4 (6%). *Lactation* ▪ (↓) bw in ♀ at lactation day 7 (5%). *Food consumption* ▪ (↓) abs (g/day) in ♂ through week 3- 5 (5-8%). ▪ (↓) abs (g/day) in ♀ at week 3 (6%). ▪ (↓) rel to bw (g/kg bw/day) in ♀ at week 3 (4%). *Necropsy findings (only ♂)* *Kidney* ▪ (↑) bilateral discoloration (13%; 4/30 animals vs 0/30 in controls). ▪ (↑) both enlarged (17%; 5/30 animals vs 0/30 in controls). ▪ (↑) both pale (3%; 1/30 animals vs 0/30 in controls). *Histopathology* *Kidney* ▪ (↑) slight/mild nephropathy in ♀ (3%; 1/30 animals vs 0/30 in controls). ▪ (↑) moderate nephropathy in ♂ (60%; 18/30 animals vs 1/30 in controls). ▪ (↑) moderate severe nephropathy in ♂ (7%; 2/30 animals vs 0/30 in controls). ▪ (↑) slight/mild tubular dilation in ♀ (3%; 1/30 animals vs 0/30 in controls). ▪ (↑) moderate tubular dilation in ♂ (50%, 15/30 animals vs 0/30 in controls). ▪ (↑) moderately severe tubular dilation in ♂ (37%; 11/30 animals vs 0/30 in controls). ▪ (↑) severe/high tubular dilation in ♂ (7%, 2/30 animals vs 0/30 in controls). ▪ (↑) slight/mild cystic tubules in ♂ (20%; 6/30 animals vs 0/30 in	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
controls).
▪ (↑) moderate cystic tubules in ♂ (13%; 4/30 animals vs 0/30 in controls).
<u>200 ppm</u> (14 ♂/ 16 ♀ mg/kg bw/day)
<u>P</u>
<u>Bodyweight and bodyweight gain</u>
Premating
▪ (↓) bw in ♀ at week 5 (5%).
▪ (↓) bwg in ♀ throughout week 0-10 (9%).
Lactation
▪ (↓) bw in ♀ at lactation days 7 (4%), 14 (4%) and 21 (5%).
<u>Food consumption</u>
▪ (↓) abs (g/day) in ♀ at week 2 (7%).
<u>Efficiency of food consumption</u>
▪ (↓) in ♂ at week 8 (25%).
<u>Histopathology</u>
Kidney
▪ (↑) moderate nephropathy in ♂ (3%; 1/30 animals vs 0/30 in controls).
<u>F1</u>
<u>Bodyweight and bodyweight gain</u>
Premating
▪ (↓) bw in ♂ through week 6-12 (5-7%).
▪ (↓) bwg in ♂ through week 10-12 (7%).
▪ (↓) bw in ♀ through week 9-12 (5-6%).
<u>Food consumption</u>
▪ (↓) abs (g/day) in ♂ at week 3 (5%), 5 (6%), 6 (8%), 9 (8%), 10 (7%), 11 (8%) and 12 (6%).
▪ (↓) abs (g/day) in ♀ at week 1 (6%), 2 (5%), 3 (8%), 6 (8%), 9 (7%) and 10 (7%).
▪ (↓) rel to bw (g/kg bw/day) in ♀ at week 3 (5%).
<u>SEXUAL FUNCTION AND FERTILITY</u>
There were no significant treatment related effects.
Summary of reproductive indices F1 generation
<table>
<tr><th>Dose (ppm)</th><th>Mating Index</th><th>Fertility Index</th><th>Gestation lenght (d)</th><th>Sex ratio (males)</th></tr>
<tr><td>0</td><td>26/30 (87%)</td><td>24/26 (92%)</td><td>22.0</td><td>48.7</td></tr>
<tr><td>200</td><td>28/30 (93%)</td><td>25/28 (89%)</td><td>21.9</td><td>45.7</td></tr>
<tr><td>2000</td><td>30/30 (100%)</td><td>29/30 (97%)</td><td>22.0</td><td>48.6</td></tr>
<tr><td>10000</td><td>26/30 (87%)</td><td>24/26 (92%)</td><td>22.0</td><td>52.9</td></tr>
</table>
Summary of reproductive indices F2 generation
<table>
<tr><th>Dose (ppm)</th><th>Mating Index</th><th>Fertility Index</th><th>Gestation lenght (d)</th><th>Sex ratio (males)</th></tr>
<tr><td>0</td><td>30/30 (100%)</td><td>26/30 (87%)</td><td>22.2</td><td>52.0</td></tr>
<tr><td>200</td><td>30/30 (100%)</td><td>29/30 (97%)</td><td>22.0</td><td>48.1</td></tr>
<tr><td>2000</td><td>27/30 (90%)</td><td>26/27 (96%)</td><td>22.0</td><td>51.6</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

10000	29/30 (97%)	29/29 (100%)	21.9	52.7

DEVELOPMENTAL TOXICITY

There were no significant treatment related effects. It has to be noted that statistical analysis on index of loss was not performed.

Summary viability data (F1a litters)

Dose (ppm)	Live born index fetuses	Liveborn[V] /stilborn pups	Total pups[V]	Live born index litters[1]	Stillborn index litters[2]	Index of loss[3]	Viability index[4]
0	95.2%	14.1/0.4	14.5	23/24 (96%)	2/24 (8%)	2/23 (9%)	23/23 (100%)
200	98.2%	14.6/0.3	14.9	25/25 (100%)	3/25 (12%)	1/25 (4%)	25/25 (100%)
2000	95.6%	13.0/0.4	13.4	29/29 (100%)	8/29 (28%)	1/29 (3%)	29/29 (100%)
10000	98.7%	13.4/0.2	13.6	24/24 (100%)	2/24 (8%)	5/24 (21%)	24/24 (100%)

[1] No. litters with live pups (day 0) / total no. litters
[2] No. litters with stilborn pups / total no. litters
[3] No. litters losing one o more pups day 0 to day 21 /No. litters day 0
[4] No. litters with live pups day 21 / No. litters with live pups day 0
[V] Bartlett's test significant. Accoding to the original reference non-parametric procedure was used and no significance was reported.

Summary viability data (F2a litters)

Dose (ppm)	Live born index fetuses	Liveborn[V] /stilborn pups	Total pups[V]	Live born index litters[1]	Stillborn index litters[2]	Index of loss[3]	Viability index[4]
0	97.1%	12.7/0.5	13.1	26/26 (100%)	6/26 (23%)	3/26 (12%)	26/26 (100%)
200	96.6%	13.7/0.5	14.2	29/29 (100%)	8/29 (28%)	4/29 (14%)	29/29 (100%)
2000	97.6%	13.1/0.4	13.5	25/25 (100%)	7/25 (28%)	1/25 (4%)	25/25 (100%)
10000	97.5%	12.4/0.3	12.7	29/29 (100%)	6/29 (21%)	5/29 (17%)	28/29 (97%)

[1] No. litters with live pups (day 0) / total no. litters
[2] No. litters with stilborn pups / total no. litters
[3] No. litters losing one o more pups day 0 to day 21 /No. litters day 0
[4] No. litters with live pups day 21 / No. litters with live pups day 0
[V] Bartlett's test significant. Accoding to the original reference non-parametric procedure was used and no significance was reported.

F_1

10000 ppm (717 ♂/801 ♀ mg/kg bw/day)
- (↓) bw at day 7 (10%), 14 (15%) and 21 (18%).

F_2

10000 ppm (717 ♂/801 ♀ mg/kg bw/day)
- (↓) bw at day 0 (8%), 7 (14%), 14 (15%) and 21 (20%).

NOAEL developmental toxicity: set in RAR (2016) **2000 ppm** (141 and 160 mg/kg bw/day for males and females) based on the decreased pups weights noted in F_1 and F_2 litters during lactation at 10000 ppm dose group. However, this decrease is regarded in this CLH Report of doubtful toxicological relevance due to the presence of maternal toxicity as it is further discussed in section 10.10.6.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		NOAEL sexual function and fertility**: 10000 ppm** (717 and 801 mg/kg bw/day for males and females) since no effects on sexual function and fertility were detected at any dose level. **NOAEL** parental toxicity: **200 ppm** (14 and 16 mg/kg bw/day for males and females) based on the kidney alterations observed at 2000 ppm dose groups.	

Table 66: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 67: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The potential effects of flazasulfuron on sexual function and fertility have been investigated in two generational studies in rat; a one -generation range-finding study (Anonymous 23, 1993; B.6.6.1.1) and a 2-generation study (Anonymous 24, 1995b; B.6.6.1.2). These studies were performed prior to the current OECD TG 416 (2001), and they were therefore deficient in some endpoints including sperm analysis, quantitative evaluation of primordial follicles or organ weights. However, the two-generation study is considered acceptable for the evaluation of sexual function and fertility assessment for the tested parameters.

The range-finding study in rats (Anonymous 23, 1993; B.6.6.1.1) was designed to select the appropriate dose levels for a further two-generation reproduction study. This was done by assessing the effects of flazasulfuron at various dose levels on parental animals and on reproduction through a single generation. Thus, test substance was tested at dose levels of 0, 100, 500, 2500 and 10000 ppm (equivalent to 0, 7, 34, 175 and 731 mg/kg bw/day for males and 0, 8, 40, 196 and 791 mg/kg bw/day for females, respectively), in which 16 pairs of males and females were mated from each test group. In the 2500 ppm group, only 15 pairs were mated due to a male death on the week 2 of the study.

Neither mortality or morbidity symptoms, nor clinical signs were detected in any animal during test substance administration.

At 10000 ppm dose group, statistically significantly lower values in bodyweight (16-17% in males and 10-12% in females) and bodyweight gain (25% in males and 27% in females) were noted throughout the premating period. On the other hand, at 2500 ppm dose group, the male mean bodyweight (7-10%) and bodyweight gains (14%) were also lower than the control values. For females, the difference in mean bodyweight did not reach

statistical significance for any week, but the mean bodyweight gain was statistically lower (15%) throughout the premating period.

Table 68: Mean Male Body Weights (g)

Group No.	Dose, ppm	Week 0	Week 1^{++}	Week 4^{++}	Week 7^{++}	Week 10^{++}	Week ^{V}14^{++}	Week ^{V}18^{++}
1	0	214.3 (15.2) N = 16	274.3 (14.7) n = 16	424.5 (19.3) n = 16	506.1 (27.5) n = 16	555.4 (32.9) n = 16	605.1 (30.9) n = 16	650.8 (36.3) n = 16
2	100	205.3 (16.7) n = 16	262.8 (19.8) N = 16	405.2 (43.7) n = 16	484.1 (62.8) n = 16	536.1 (73.2) n = 16	583.3 (81.9) n = 16	629.8 (92.8) n = 16
3	500	210.6 (13.1) n = 16	268.0 (16.1) n = 16	409.2 (32.1) n = 16	481.3 (49.9) n = 16	535.4 (47.1) n = 16	586.9 (49.2) n = 16	631.0 (52.6) n = 16
4	2500	207.0 (14.5) n = 16	**255.2* (18.0)** **n = 16**	**390.3* (29.2)** **n = 16**	463.0 (44.7) n = 15	517.1 (47.0) n = 15	565.0 (52.0) n = 15	603.4 (62.3) n = 15
5	10000	209.7 (13.2) n = 16	**229.0** (19.6)** **n = 16**	**358.3** (35.3)** **n = 16**	**419.8**(43.5)** **n = 16**	**466.6** (45.7)** **n = 16**	**511.4** (55.3)** **n = 16**	**552.0** (53.0)** **n = 16**

V= Bartlett´s test significant.
**Statistically sifnificant difference from control group at the 0.01 level,
++ Statistically significant trend at the 0.01 level

Table 69: Mean Female Body Weights (g)

Group No.	Dose, ppm	Week 0	Week 2^{++}	Week 4^{++}	Week 7^{++}	Week 10^{++}
1	0	167.8(13.0) N = 16	220.6(21.3) n = 16	260.9(24.2) n = 16	289.3(25.2) n = 16	309.3(26.1) n = 16
2	100	169.2(13.2) n = 16	221.2(18.5) n = 16	262.3(26.7) n = 16	292.0(26.3) n = 16	309.8(29.8) n = 16
3	500	169.9(9.6) n = 16	224.1(18.4) n = 16	266.5(25.5) n = 16	302.4(29.7) n = 16	324.9(34.6) n = 16
4	2500	165.6(13.7) n = 16	206.6(18.0) n = 16	241.6(23.1) n = 16	270(24.1) n = 16	286.3(23.6) n = 16
5	10000	168.6(12.4) n = 16	**199.2**(15.3)** **n = 16**	**231.7** **(19.1)** **n = 16**	**255.6** **(18.6)** **n = 16**	**272.1** **(22.1)** **n = 16**

$^{++}$ Statistically significant trend, p = < 0.001
* Statistically significant difference from control group p = < 0.05
** Statistically significant difference from control group p = < 0.01

Statistically significant decrease in dams bodyweights were recorded in the high dose group throughout whole gestation period (13-15%), whereas in the 2500 ppm dose group these differences were noted from gestation day 7 (7-8%). In lactation period, 10000 ppm dams exhibited statistically significant reduction in bodyweight throughout entire period (7-14%).

Food consumption was measured only during premating period. Statistically significant decrease in absolute food consumption (g/day) was only observed in the high dose groups for both sexes (14-16% for males and 9-

15% for females, respectively). No remarkable differences were observed in food consumption relative to bodyweight.

Reproduction parameters such as mating, fertility index, sex ratio or gestation length were not affected by treatment. The reduction of mating index at 10000 ppm (81%) compared to controls (100%) was regarded not relevant since it was not significant and not dose-dependent. Number of implantations, corpora lutea, post-implantation loss, sperm parameters and sexual organ weights were not measured, and histopathological examination in sexual organs was not performed. The mean litter size of live born pups was 16.5, 15.4, 14.2, 13.8 and 13.3 in the control, 100, 500, 2500 and 10000 ppm dose groups, respectively, and due to lack of data on number of implantations, it is not possible to determine whether this reflects the reduced number of implantations (to be addressed under sexual function and fertility) or postimplantation losses (to be addressed under developmental toxicity) or both. As noted, the mean number of live born pups on lactation day 0 was lower in all the treatment groups compared with control group, and showed a dose-related trend. However, the only value that displayed a statistical significant difference was the mean litter size of live born pup of 2500 ppm dose group. Historical control data (HCD) from three previous studies conducted in same institute between 1987 and 1991 displayed a live born pups range of 11.3 to 14.5. Three more studies were further conducted between 1992 and 1996, and the mean litter size of live born pup ranged from 12.7 to 14.1. Thus, it is important to remark that the live born pup mean of control group (16.5) and 100 ppm dose group (15.4) were slightly higher than observed in HCD. Therefore, the differences in the number of live born pups between the treatment groups and the control group were considered as incidental nature and no treatment-related.

NOAEL for **sexual function and fertility** could be established at **10000 ppm (731 / 791 mg/kg bw/day for males / females)** since no effects on sexual function and fertility were detected at any dose level.

NOAEL for **parental toxicity** could be established at **500 ppm (34 / 40 mg/kg bw/day for males / females)** based on the decreased bodyweight and bodyweight gains observed at 2500 ppm dose groups in both sexes.

In the two-generation reproductive study in rats (Anonymous 24, 1995b; B.6.6.1.2), flazasulfuron was tested at 0, 200, 2000 and 10000 ppm dose levels (equivalent to 0, 14, 141, and 717 mg/kg bw/day for males and 0, 16, 160, 801 mg/kg bw/day for females, respectively), in which 30 pairs of males and females were mated from each test group. The selection of doses in this study seems to be correct regarding the effects observed in the previous preliminary study (Anonymous 23, 1993; B.6.6.1.1).

Neither gross mortality or morbidity signs, nor treatment related clinical signs were observed in any dose group. Few sporadic animals were found dead or humanely killed. These deaths were not considered related to treatment. Throughout premating period (growth phase), statistically significant differences (>10%) in bodyweights and bodyweight gain were recorded for both sexes in parental P and F_1 animals at 10000 ppm dose group. In addition, reduced bodyweights (>10%) were subsequently described throughout whole gestation and lactation periods in both P and F_1 parental dams at high dose groups.

At 2000 ppm dose group, bodyweights and bodyweight gain were statistically significantly lower in P male animals throughout the ten weeks of growth period (5-6% and 9%, respectively). On the other hand, P females did not show differences in absolute bodyweight throughout premating period, however, bodyweight gain was reduced 8% compared with control group. Additionally, dams from P generation exhibited lower bodyweights from lactation day 7 (5-6%). Regarding F_1 animals of mid dose group, males exhibited reduced bodyweights during the first 6 weeks of premating period (5-6%). Moreover, F_1 dams showed reduced bodyweight at lactation day 7 (5%), but no further reductions in bodyweight were recorded during other days of lactation or gestation period.

At 200 ppm group, only P females displayed low bodyweight gain throughout premating period (9%) compared with control group. In addition, decreased bodyweight were noted from lactation day 7 (4-5%). In F_1 parental animals, reduced bodyweights and bodyweights gain were noted during the last month of the premating period (5-7%). Taking into account the low magnitude of the reductions the decreases are not considered adverse.

Table 70: Summary of Body Weights from Selected Weeks (Expressed As % Of Control Values)

F0 generation, % of control weight				
Dose, ppm	**Sex**	**Week 1**	**Week 5**	**Week 10**
200	M	99.8	100.3	99.2
2000	M	95.4**	94.3**	94.6*
10000	M	85.4**	85.8**	83.1**
200	F	97.7	95.4*	95.6
2000	F	97.9	96.7	97.1
10000	F	91.2**	86.5**	87.2**

* Statistically significant difference from control group p = < 0.05
** Statistically significant difference from control group p = < 0.01

F1 parental animal findings:

Table 71: Summary of Body Weights from Selected Weeks (Expressed as % of Control Values)

F1 generation, % of control weight				
Dose, ppm	**Sex**	**Week 1**	**Week 5**	**Week 10**
200	M	95.3	95.9	94.1*
2000	M	94.7*	94.9*	95.4
10000	M	76.2**	78.5**	78.3**
200	F	96.0	94.9	94.3*
2000	F	96.1	97.4	99.0
10000	F	82.9**	84.1**	84.8**

* Statistically significant difference from control group p = < 0.05
** Statistically significant difference from control group p = < 0.01

Absolute food consumption (g/day) was statistically significantly lower in 10000 ppm P males (14-43%) and females (7-35%) during premating period. Similar results were observed in F_1 males (19-21%) and females (12-17%). However, food consumption relative to bodyweight was statistically significantly higher in P and F_1 males almost during the whole premating period (3-6%).

At 2000 ppm dose group, absolute food consumption was consistently lower in P and F_1 males (5-9%), although the differences appeared to occur mostly during the first month of the growth period. In females, absolute and relative food consumption were similar to controls throughout both generations.

At 200 ppm dose group, absolute food consumption was lower in both F_1 males and females during most weeks of the premating period, however, the differences were less than 10% and, therefore, were not considered to be biologically significant.

Efficiency of food consumption showed statistically lower mean values at high dose male and female groups during the first weeks of the P generation growth phase. At week 1, these differences reached the 58% and 62% in males and females, respectively. Moreover, F_1 parental males exhibited reduced food consumption efficiency during first weeks of the premating period (4-9%). These lower values may have been influenced by increases in spillage of food in some of the animals, which it is often an indication that the animals do not find the food palatable.

At 2000 ppm dose group, efficiency of food consumption also reduced at first week in both sexes in P animals (15% and 19% for males and females, respectively). No relevant differences were noted in F_1 parental animals or in low dose groups.

Increased spillage food observations were detected in the first week of P generation in the high and mid dose groups. Authors considered that the reduced food consumption values during the first weeks of premating period of P generation may be partially due to spillage and may not accurately indicate consumption by the animals. Consequently, the data for efficiency of food utilisation suggests that the animals from each of the groups utilised approximately the same amount of feed to gain a set amount of bodyweight. This suggests that the lower bodyweights are due to reduced food consumption, probably due to reduced palatability of the feed.

The paragraphs summarized in section 10.10.5 present the results of necropsy examinations and histopathological analysis in which kidney of P and F1 animals (mainly males) was apparently affected by treatment. Effects in P animals are univocally relevant for STOT RE. In the case of F1 animals reasonable doubts arises if they are relevant for development or STOT RE. However, taking into account that these effects are post mortem examinations after dosing during premating and also gestation and lactation (long dosing periods) in our opinion they are relevant for STOT RE but not for development.

Reproduction parameters such as mating, fertility index, sex ratio or gestation length were not affected by treatment. Fertility indices (no. of females pregnant/no. females with positive signs of mating) for control, 200 ppm, 2000 ppm and 10000 ppm dose groups in the F_1 generation were 92%, 89%, 97% and 92% and in the F2 generation 84%, 97%, 96% and 100% indicating no treatment-related effects. Quatitative evaluation of implantation sites, corpora lutea, sexual organ weights (including uterus, ovaries, testes, epididymides, prostate, seminal vesicles), sperm parameters and primordial follicles was not performed..

NOAEL for **sexual function and fertility** has been established at **10000 ppm (717 / 801 mg/kg bw/day for males / females)** since no effects on sexual function and fertility were detected at any dose level.

NOAEL for **parental toxicity** has been established at **200 ppm (14 / 16 mg/kg bw/day for males / females)** based on the kidney alterations observed at 2000 ppm dose groups.

10.10.3 Comparison with the CLP criteria

For the purpose of classification for reproductive toxicity according to the criteria of the CLP (Regulation (EC) No. 1272/2008), substances are allocated to one of two categories. Within each category, effects on sexual function, fertility, lactation and development, are considered separately.

Category 1: Known or presumed human reproductive toxicant

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

Category 1A: Known human reproductive toxicant

The classification of a substance in Category 1A is largely based on evidence from humans.

Category 1B: Presumed human reproductive toxicant

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2: Suspected human reproductive toxicant

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be

the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

No human information is available on the effects of flazasulfuron on the reproductive system. Information from a reliable two-generation study in rats showed that flazasulfuron has no effects on sexual function and fertility. Both available studies did not include the no. of implantations, corpora lutea and post-implantation losses besides other parameters like a sperm analysis. However, data from both available studies is regarded conclusive for classification since sexual function and fertility parameters available such as mating and fertility indices were not affected by treatment. No significant effects were seen on number of litters in both studies and consequently it is considered that there is no potential impairment of fertility in relation with implantation.

Consequently, classification for sexual function and fertility is not warranted.

10.10.4 Adverse effects on development

Table 72: Summary table of animal studies on adverse effects on development

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>Range-finding study: A single generation reproduction study in rats

Summarised in section 10.10.1 for more detail.

Method: No guideline

GLP: Yes
Rat strain: Sprague-Dawley (CD® –Crl : (SD) BR)

Sex: ♂ and ♀

No. animals:
P: 16 rats/sex/dose.

Deviations from current test guideline (OECD TG 416, 2001):

-16 pairs of animals were used per group, instead of 20 animals at or near parturition.

-The dose interval exceed the 3 fold recommended for dietary studies.

-Food consumption not measured in reproduction phases.

-No. of implantations, corpora lutea and post-implantation loss were not measured.

-Organ weights were not recorded (uterus, ovaries, testes, epididymides, prostate, seminal vesicles, brain, liver, kidney, etc.)

Histopathological examination in sexual organs was not performed.

-Sperm parameters were not measured.</td>
<td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.1% purity.

Oral: Diet

0, 100, 500, 2500 and 10000 ppm equivalent to:
♂: 0, 7, 34, 175 and 731 mg/kg bw/day*.
♀: 0, 8, 40, 196 and 791 mg/kg bw/day*.

Exposure:
Pre-mating treatment:
P: 77 days (10 weeks)
Mating: 15 days
Treatment continued in P and F_1 throughout gestation and lactation.

Development (F_1 offspring):
Mean litter size, pup sex ratios, number of live and stillborn pups at birth, day 4 pup viability index, lactation index, pup wt, external abnormalities.

*Equivalences are based on mean compound consumption throughout premating period (10 weeks). Equivalences in RAR 2016 are only based on week 10.</td>
<td>The study has been previously summarised in section 10.10.2 in case of more detail is needed. Only effects on development are included in this table.

DEVELOPMENTAL TOXICITY (F_1)
It has to be noted that the effects on live born pups could be associated to developmental or fertility impairment since the no. of implantations was not measured.

10000 ppm (731 ♂/ 791 ♀ mg/kg bw/day)
▪ (↓) live born pups (19% but not statistically significant).
▪ (↓) total pups (19%, statistically significant).
▪ (↓) bw at day 14 (7%) and 21 (10%).

Mean live born pups per litter
<table>
<tr><th>HCD (1987-1991) study</th><th>year</th><th>Range</th></tr>
<tr><td>1722-87-0121-TX-003</td><td>1987</td><td rowspan="3">11.3-14.5</td></tr>
<tr><td>1703-88-0176-TX-003</td><td>1988</td></tr>
<tr><td>3822-91-0014-TX-003</td><td>1991</td></tr>
<tr><th>Dose (ppm)</th><th>year</th><th colspan="2">mean live born pups (no.litters)</th></tr>
<tr><td>0</td><td rowspan="5">1993</td><td colspan="2">16.5 (16)</td></tr>
<tr><td>100</td><td colspan="2">15.4 (12)</td></tr>
<tr><td>500</td><td colspan="2">14.2 (14)</td></tr>
<tr><td>2500</td><td colspan="2">13.8* (15)</td></tr>
<tr><td>10000</td><td colspan="2">13.3 (12)</td></tr>
<tr><th>HCD (1992-1996) study</th><th>year</th><th>mean live born pups (no.litters)</th><th>Range</th></tr>
<tr><td>5330-92-0223-TX-004</td><td>1992</td><td>F1= 14.1 F2=12.7</td><td rowspan="3">12.7-14.1</td></tr>
<tr><td>6755-96-0042-TX-003</td><td>1992</td><td>F1= 13.6 F2=13</td></tr>
<tr><td>6754-96-0041-TX-001</td><td>1996</td><td>13.6</td></tr>
</table>
*p<0.05 from control group in the same study.

500 ppm (34 ♂/ 40 ♀ mg/kg bw/day)
▪ (↑) bw at day 21 (8%, ndr).

NOAEL developmental toxicity: set in RAR (2016) at 2500 ppm (175 and 196 mg/kg bw/day for males and females) based on the decreased pups weight noted on lactation days 14 and 21 at 10000 ppm dose group. However, this decrease is regarded in this CLH Report of doubtful toxicological relevance due to the presence of maternal toxicity as it is further discussed in section 10.10.6.</td>
<td>Anonymous 23 (1993) (CA) B.6.6.1.1</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td>-Macroscopic examination and gross necropsy were not conducted.

Acceptable as preliminary study</td><td></td><td></td><td></td></tr>
<tr><td>A two- generation reproduction study in rats

Summarised in section 10.10.1 for more detail.

Method: US EPA 83-4 comparable to OECD TG 416 (1983)

GLP: Yes
Rat strain: Sprague-Dawley (CD® –Crl : (SD) BR)

Sex: ♂ and ♀

No. animals:
P: 30 rats/sex/dose.

Deviations from current test guideline (OECD TG 416, 2001):

-The dose interval exceed the 3 fold recommended for dietary studies.

-Food consumption not measured during reproductive phases.

-No. of implantations, corpora lutea and post-implantation loss were not analysed.

-Organ weights were not recorded (uterus, ovaries, testes, epididymides, prostate, seminal vesicles, brain, liver, kidney, etc.)

-Sperm parameters were not measured.

-Quantitative evaluation of primordial follicles was not performed.</td><td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.1% purity.

Oral: Diet
0, 200, 2000, and 10000 ppm equivalent to:
♂: 0, 14, 141, and 717 mg/kg bw/day*.
♀: 0, 16, 160, and 801 mg/kg bw/day*.

Exposure:

Pre-mating treatment:
P: 77 days (10 weeks)
F_1: 84 days (12 weeks)
P and F_1 mating : 15 days
Treatment continued in P, F_1 and F_2 throughout gestation and lactation.

Parameters observed

P/F_1 parental animals:

Mortality, clinical signs, bw, bw gain and food consumption.

F_1/F_2 offspring:

Mean litter size, pup sex ratios, number of live and stillborn pups at birth, day 4 pup viability index, lactation index, pup wt, external abnormalities, and histopathological analysis.

*Equivalences are based on mean compound consumption throughout F_1 and F_2 premating period (10</td><td>The study has been previously summarised in section 10.10.2 in case of more detail is needed. Only effects on development are included in this table.

DEVELOPMENTAL TOXICITY

There were no significant treatment related effects. It has to be noted that statistical analysis on index of loss was not performed.

Summary viability data (F1a litters)
<table>
<tr><th>Dose (ppm)</th><th>Live born index fetuses</th><th>Liveborn[V] /stilborn pups</th><th>Total pups[V]</th><th>Live born index litters[1]</th><th>Stillborn index litters[2]</th><th>Index of loss[3]</th><th>Viability index[4]</th></tr>
<tr><td>0</td><td>95.2%</td><td>14.1/0.4</td><td>14.5</td><td>23/24 (96%)</td><td>2/24 (8%)</td><td>2/23 (9%)</td><td>23/23 (100%)</td></tr>
<tr><td>200</td><td>98.2%</td><td>14.6/0.3</td><td>14.9</td><td>25/25 (100%)</td><td>3/25 (12%)</td><td>1/25 (4%)</td><td>25/25 (100%)</td></tr>
<tr><td>2000</td><td>95.6%</td><td>13.0/0.4</td><td>13.4</td><td>29/29 (100%)</td><td>8/29 (28%)</td><td>1/29 (3%)</td><td>29/29 (100%)</td></tr>
<tr><td>10000</td><td>98.7%</td><td>13.4/0.2</td><td>13.6</td><td>24/24 (100%)</td><td>2/24 (8%)</td><td>5/24 (21%)</td><td>24/24 (100%)</td></tr>
</table>
[1] No. litters with live pups (day 0) / total no. litters
[2] No. litters with stilborn pups / total no. litters
[3] No. litters losing one o more pups day 0 to day 21 /No. litters day 0
[4] No. litters with live pups day 21 / No. litters with live pups day 0
[V] Bartlett's test significant. Accoding to the original reference non-parametric procedure was used and no significance was reported.

Summary viability data (F2a litters)
<table>
<tr><th>Dose (ppm)</th><th>Live born index fetuses</th><th>Liveborn[V] /stilborn pups</th><th>Total pups[V]</th><th>Live born index litters[1]</th><th>Stillborn index litters[2]</th><th>Index of loss[3]</th><th>Viability index[4]</th></tr>
<tr><td>0</td><td>97.1%</td><td>12.7/0.5</td><td>13.1</td><td>26/26 (100%)</td><td>6/26 (23%)</td><td>3/26 (12%)</td><td>26/26 (100%)</td></tr>
<tr><td>200</td><td>96.6%</td><td>13.7/0.5</td><td>14.2</td><td>29/29 (100%)</td><td>8/29 (28%)</td><td>4/29 (14%)</td><td>29/29 (100%)</td></tr>
<tr><td>2000</td><td>97.6%</td><td>13.1/0.4</td><td>13.5</td><td>25/25 (100%)</td><td>7/25 (28%)</td><td>1/25 (4%)</td><td>25/25 (100%)</td></tr>
<tr><td>10000</td><td>97.5%</td><td>12.4/0.3</td><td>12.7</td><td>29/29 (100%)</td><td>6/29 (21%)</td><td>5/29 (17%)</td><td>28/29 (97%)</td></tr>
</table>
[1] No. litters with live pups (day 0) / total no. litters
[2] No. litters with stilborn pups / total no. litters
[3] No. litters losing one o more pups day 0 to day 21 /No. litters day 0
[4] No. litters with live pups day 21 / No. litters with live pups day 0
[V] Bartlett's test significant. Accoding to the original reference non-parametric procedure was used and no significance was reported.

F_1

10000 ppm (717 ♂/801 ♀ mg/kg bw/day)
▪ (↓) bw at day 7 (10%), 14 (15%) and 21 (18%).

F_2

10000 ppm (717 ♂/801 ♀ mg/kg bw/day)
▪ (↓) bw at day 0 (8%), 7 (14%), 14 (15%) and 21 (20%).</td><td>Anonymous 24 (1995b) (CA) B.6.6.1.2</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td>Acceptable study</td><td>weeks in F1 and 12 weeks in F2 generations). Equivalences in RAR 2016 are only based on week 10 of F1 generation.</td><td>NOAEL developmental toxicity: set in RAR (2016) 2000 ppm (141 and 160 mg/kg bw/day for males and females) based on the decreased pups weights noted in F1 and F2 litters during lactation at 10000 ppm dose group. However, this decrease is regarded in this CLH Report of doubtful toxicological relevance due to the presence of maternal toxicity as it is further discussed in section 10.10.6.</td><td></td></tr>
<tr><td>Pilot study: Teratogenicity study in rats

Method: No guideline, range-finding study

GLP:Yes

Rat strain: Wistar-Imamichi rats

Sex: 8 mated females/group.

Deviations from current test guideline (OECD TG 414, 2018):

-Groups did not receive the same volume of test chemical.

-The exposure period was from day 6 to 15 of gestation instead of the recommended period from implantation (e.g. day 5 post mating) to the day prior to scheduled caesarean section.

-The following endpoints were not measured:

-Wt of the gravid uteri including cervix, anogenital distance, thyroid weight, thyroid hormones (T3/T4/TSH), indication of incomplete testicular descent/cryptorchidism.

-Only dose groups of 0, 50 and 1000 mg/kg bw/day were examined for visceral and skeletal malformations. It has to be noted that half of the fetuses were examined for skeletal malformations and the</td><td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 96.3% purity.

Oral gavage

0, 50, 200, 500 and 1000 mg/kg bw/day from day 6 to 15 of pregnancy both included
Litter data not available

Parameters observed:

Maternal data:

Clinical signs, mortality, bw and bwg, food consumption.

Reproductive data:

Number (no.) of corpora lutea, no. implants, no. resorptions, placental wt, no. live and dead foetuses, no. dams with externally abnormal foetuses

Foetal data:

Foetus wt, foetus sex and foetus alterations (external, visceral and skeletal)</td><td>Maternal toxicity

Mortality: no mortality or morbidity signs were observed in females at any dose level.

Clinical signs: No treatment related clinical signs were observed.

1000 mg/kg bw/day
Bodyweight and bodyweight gain:
▪ (↓) bw on days 9 (5%), 12 (6%) and 18 (6%).
▪ (↓) bwg through days 6-9 (145%).
▪ (↓) food consumption on days 6-9 (37%) and 9-12 (30%).
Food consumption:
▪ (↓) through days 6-9 (37%) and 9-12 (30%),

500 mg/kg bw/day
Bodyweight and bodyweight gain:
▪ (↓) bwg through days 6-9 (64%).

Developmental toxicity
<table>
<tr><th>Findings</th><th>0 mg/kg bw</th><th>50 mg/kg bw</th><th>200 mg/kg bw</th><th>500 mg/kg bw</th><th>1000 mg/kg bw</th></tr>
<tr><td>No. of dams</td><td>8</td><td>8</td><td>7</td><td>8</td><td>8</td></tr>
<tr><td>No. of corpora lutea</td><td>18.6</td><td>18.4</td><td>17.9</td><td>18.4</td><td>17.5</td></tr>
<tr><td>No. of implants</td><td>15.6</td><td>14.8</td><td>15.7</td><td>15.6</td><td>14.4</td></tr>
<tr><td>Resorptions
Early
Late
Total</td><td>
5
1
6</td><td>
8
2
10</td><td>
3
1
4</td><td>
8
0
8</td><td>
21
1
22</td></tr>
<tr><td>Foetal mortality (early and late resorptions)</td><td>4.8%</td><td>8.4%</td><td>3.5%</td><td>6.1%</td><td>19.4%</td></tr>
<tr><td>Total number of live foetuses</td><td>119</td><td>108</td><td>106</td><td>117</td><td>93</td></tr>
<tr><td>No. of foetuses examined for:
Skeletal malformations
Visceral malformations</td><td>
62
57</td><td>
56
52</td><td>
-
-</td><td>
-
-</td><td>
49
44</td></tr>
<tr><td>No. live foetuses</td><td>14.9</td><td>13.5</td><td>15.1</td><td>14.6</td><td>11.6</td></tr>
<tr><td>Total number of litters</td><td>8</td><td>8</td><td>7</td><td>8</td><td>7</td></tr>
<tr><td>Mean foetal weight</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Female (g)</td><td>4.92</td><td>5.11*</td><td>5.03</td><td>4.99</td><td>4.72**</td></tr>
<tr><td>Male (g)</td><td>5.28</td><td>5.47</td><td>5.34</td><td>5.23</td><td>4.95**</td></tr>
</table>
Foetal body weight

1000 mg/kg bw/day
▪ (↓) ♀/♂ (4%/6%) foetal weight but ndr.

Foetal malformations and variations

Litter data given only for external malformations regarding that for skeletal and visceral malformations only half of foetuses were examined.

1000 mg/kg bw/day</td><td>Anonymous 25 (1988a) (CA) B.6.6.2.1</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td>other half for visceral malformations.

Acceptable as a preliminary study</td><td></td><td>External malformations:
▪ 1/93 foetuses (1%) / 1/7 litters (14.3%) vs 0/119 foetuses and 0/8 litters in controls (n.s.and ncdr) with preaxial polydactyly in the hind limbs
Skeletal variations:
▪ 2/49 foetuses (4%) vs 0/62 in controls (n.s.) with 14th ribs.
Visceral malformations:
▪ 3/44 foetuses (6.8%) vs 1/57 in controls (n.s.) with ventricular septal defect (VSD).

Summary of interventricular septal defect (VSD) in preliminary study
<table>
<tr><th>Finding/dose (mg/kg bw/day)</th><th>0</th><th>50</th><th>1000</th><th colspan="2">Historical control
1975-82 1983-86</th></tr>
<tr><td>Total number of foetuses examined</td><td>57</td><td>52</td><td>44</td><td>3809</td><td>2757</td></tr>
<tr><td>Total number of litters</td><td>8</td><td>8</td><td>7</td><td>NA</td><td>NA</td></tr>
<tr><td>Incidence of dams with a pup with VSD</td><td>1
(12.5%)</td><td>0
(0%)</td><td>2
(28.6%)</td><td>NA</td><td>NA</td></tr>
<tr><td>Incidence of pups with VSD</td><td>1
(1.7%)</td><td>0
(0%)</td><td>3
(6.8%)</td><td>56
(1.47%)
[0-11.3%]</td><td>42
(1.52%)
[0-5.3%]</td></tr>
</table>
(): mean incidence
[]: range

500 mg/kg bw/day
External malformation:
▪ 1/117 live foetuses (0.8%) / 1/8 (12.5%) in litters vs 0/119 foetuses and 0/8 inlitters in controls (n.s.; ndr) with vestigial tail and anal atresia.

50 mg/kg bw/day

External malformations
▪ 1/108 foetuses (0.9%)/ 1/8 litters (12.5%) vs 0/119 foetuses and 0/8 litters in controls (n.s.; ndr) edema, micrognathia, cleft palate, preaxial polydactyly .
Skeletal malformations
▪ 1/56 foetuses (1.7%) vs 0/62 in controls (n.s.; ndr) splitting of 1st and 2nd sternebrae and fusion of 3rd-4th-5th sternebrae.
Visceral malformations:
▪ 1/52 foetuses (1.9%) vs 0/57 in controls (n.s.; ndr) with dilation of renal pelvis and ureter.</td><td></td></tr>
<tr><td>Main study: Teratogenicity study in rats

Method: OECD TG 415 (1983) and US EPA 83-3

GLP:Yes

Rat strain: Wistar-Imamichi rats

Sex: 23 mated females/group.

Deviations from</td><td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 96.3% purity.

Oral gavage

0, 100, 300 and 1000 mg/kg bw/day from day 6 to 15 of</td><td>Maternal toxicity

Mortality: no mortality or morbidity signs were observed in females at any dose level.

Clinical signs: No treatment related clinical signs were observed.

1000 mg/kg bw/day
Bodyweight and bodyweight gain:
▪ (↓) bw on day 9 (6%), 12 (6%), 15 (7%), 18 (7%) and 21 (8%).
▪ (↓) bwg through days 6-9 (150%) and 12-15 (21%).

Food consumption:
▪ (↓) through days 6-9 (36%), 9-12 (23%), 12-15 (20%) and (↑) through days 18-21 (13%, ndr).

300 mg/kg bw/day</td><td>Anonymous 26 (1988b) (CA) B.6.6.2.2</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>current test guideline (OECD TG 414, 2018):

-The exposure period was from day 6 to 15 of gestation instead of the recommended period from implantation (e.g. day 5 post mating) to the day prior to scheduled caesarean section.

-The following endpoints were not measured:

-Wt of the gravid uteri including cervix, anogenital distance, thyroid weight, thyroid hormones (T3/T4/TSH).

Half of the fetuses were examined for skeletal malformations and the other half for visceral malformations.

Acceptable study</td>
<td>pregnancy both included

Parameters observed:

Maternal data:

Clinical signs, mortality, bw and bwg, food consumption.

Reproductive data:

Number (no.) of corpora lutea, no. implants, no. resorptions, placental wt, no. live and dead foetuses, no. dams with externally abnormal foetuses

Foetal data:

Foetus wt, foetus sex and foetus alterations (external, visceral and skeletal)</td>
<td>Bodyweight and bodyweight gain:
▪ (↓) bwg through days 6-9 (50%).

Food consumption:
▪ (↓) through days 6-9 (11%).

Developmental toxicity
<table>
<tr><th>Findings</th><th>0 mg/kg bw</th><th>100 mg/kg bw</th><th>300 mg/kg bw</th><th>1000 mg/kg bw</th></tr>
<tr><td>No. of dams</td><td>23</td><td>23</td><td>23</td><td>22</td></tr>
<tr><td>No. of corpora lutea</td><td>17.5</td><td>16.6</td><td>18.0</td><td>17.5</td></tr>
<tr><td>No. of implants</td><td>14.9</td><td>14.4</td><td>14.2</td><td>14.8</td></tr>
<tr><td>Resorptions
Early
Late
Total</td><td>
15
4
19</td><td>
11
1
12</td><td>
10
6
16</td><td>
59
10
69</td></tr>
<tr><td>Foetal mortality (early and late resorptions)</td><td>5.5%</td><td>3.7%</td><td>4.7%</td><td>20.8%</td></tr>
<tr><td>Total number of live foetuses</td><td>323</td><td>320</td><td>310</td><td>256</td></tr>
<tr><td>No. of foetuses examined for:
Skeletal malformations
Visceral malformations</td><td>
167
153</td><td>
167
153</td><td>
131
149</td><td>
134
122</td></tr>
<tr><td>No. live foetuses</td><td>14.0</td><td>13.9</td><td>13.5</td><td>11.6</td></tr>
<tr><td>Total number of litters</td><td>23</td><td>23</td><td>23</td><td>20</td></tr>
<tr><td colspan="5">Mean foetal weight</td></tr>
<tr><td>Female (g)</td><td>5.15</td><td>5.16</td><td>5.17</td><td>4.73** (8%)</td></tr>
<tr><td>Male (g)</td><td>5.52</td><td>5.55</td><td>5.55</td><td>5.09** (8%)</td></tr>
</table>
Foetal deaths

1000 mg/kg bw/day
▪ (↑) foetal deaths (20.8% vs 5.8% in controls; n.s.).

Foetal body weight
1000 mg/kg bw/day
▪ (↓) ♀/♂ (8%/8%) foetal weight.

Foetal malfomations and variations:
Litter data given only for external malformations regarding that for skeletal and visceral malformations only half of foetuses were examined.

1000 mg/kg bw/day
External malformations:
▪ 1/256 (0.4%) foetuses / 1/20 (5%) litters vs 0/323 foetuses and 0/23 litters in controls (n.s.; ncdr) with short trunk (due to the absence of the lumbar, sacral and caudal vertebrae), vestigial tail and anal atresia.
▪ 1/256 (0.4%) foetuses / 1/20 (5%) litters vs 0/323 foetuses and 0/23 litters in controls (n.s.; ncdr) with short trunk (due to the absence of the lumbar, sacral and caudal vertebra), tailless, anal atresia and clubfoot.

Skeletal malformations:
▪ 1/134 (0.7%) foetuses vs 0/167 in controls (n.s.; ncdr) with fusion of all the sternebrae, absence of vertebrae below 2nd lumber</td>
<td></td>
</tr>
</table>

vertebra, absence of ribs below 2nd ribs, and absence of coxa.

Skeletal variations:

- 12/134 (1.5%) foetuses vs 0/167 in controls (n.s.; ncdr) with splitting or dumbbell -shape of thoracic vertebral body.
- 15/134 (11.2%) foetuses vs 0/167 in controls with 14th rib.

Incidence of 14th extra rib

Finding/dose (mg/kg bw/d)	0	100	300	1000	Historical control 1975-82	Historical control 1983-86
Total number of foetuses examined	167	167	161	134	18901	3452
Total number of litters	23	23	23	20	NA	NA
Incidence of dams with a pup with extra rib	0	0	1 (4.3%)	5 (25%)	NA	NA
Incidence of pups with an extra rib	0	0	2 (1.2%)	15* (11.2%)	128 (0.68%)	21 (0.61%) [0-3.1%]

**Statistically significant p = <0.05*
(): mean incidence
[]: range

- (↓) foetuses with 5 ossified metatarsals (72.8% vs 99.4% in controls).

Visceral malformations:

- 8/122 (6.6%) vs 2/156 (1.3%) in controls with ventricular septal defect (VSD).
- 2/122 (1.6%) foetuses vs 0/156 in controls (n.s.) with dilation of renal pelvis and ureter.

Summary of interventricular septal defect (VSD)

Finding/dose (mg/kg bw/d)	0	100	300	1000	Historical control 1975-82	Historical control 1983-86
Total number of foetuses examined	156	153	149	122	3809	2757
Total number of litters	23	23	23	20	NA	NA
Incidence of dams with a pup with VSD	1 (4.3%)	1 (4.3%)	6 (26%)	7 (35%)	NA	NA
Incidence of pups with VSD	2 (1.3%)	1 (0.7%)	6* (4.0%)	8* (6.6%)	56 (1.47%) [0-11.3%]	42 (1.52%) [0-5.3%]

**Statistically significant p = <0.05*
(): mean incidence
[]: range

300 mg/kg bw/day

Skeletal variation:

- 2/161 (1.2%) foetuses examined vs 0/167 in controla (n.s.) with 14th rib.

Visceral malformations:

- 6/149 (4%) foetuses vs 2/156 in controls with ventricular septal

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>defect (VSD).
▪ 1/149 (0.7%) foetuses vs 0/156 in controls (n.s.) with dilation of renal pelvis and ureter
100 mg/kg bw/day
Visceral malformations:
▪ 1/153 (0.7%) foetuses vs 2/156 in controls (n.s) with ventricular septal defect (VSD).
NOAEL developmental toxicity: 300 mg/kg bw/day based on foetal deaths and delayed in ossified metatarsals observed at 1000 mg/kg bw/day.
NOAEL maternal toxicity: 100 mg/kg bw/day based on decreased bodyweight gain and low food consumption rates detected in dams at 300 mg/kg bw/day.</td><td></td></tr>
<tr><td>Developmental toxicity study in rats

Method; OECD TG 414 (1981) and US EPA 83-3.

GLP:Yes

Rat strain: Sprague-Dawley (CD® –Crl : (SD) BR)

Sex: 24 mated females/group.

Deviations from current test guideline (OECD TG 414, 2018):

-The exposure period was from day 6 to 15 of gestation instead of the recommended period from implantation (e.g. day 5 post mating) to the day prior to scheduled caesarean section.

-The following endpoints were not measured:

Thyroid weight, thyroid hormones (T3/T4/TSH), and anogenital distance.

Acceptable study</td><td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.3% purity.

Oral gavage

0, 100, 300 and 1000 mg/kg bw/day from day 6 to 15 of pregnancy both included

Parameters observed:

Maternal data:

Mortality, clinical signs, bw and bwg, food consumption, liver wt.

Reproductive data:

Gravid uterine wt, no. corpora lutea, no. implants, no. resorptions (late and early), no. litters with resorptions, nº of pregnancies dams, nº aborted, no. live and dead foetuses

Foetal data:

Foetus wt, foetus sex and foetus alterations (external, visceral and skeletal)</td><td>Maternal toxicity

Mortality: no mortality or morbidity signs were observed in females at any dose level.

Clinical signs: Increased anogenital stains (25% vs 4% in controls) and alopecia observations (16% vs 0% in controls) at 1000 mg/kg bw day dose group.

1000 mg/kg bw/day
Bodyweight and bodyweight gain:
▪ (↓) bw on day 9 (6%), 12 (6%), 16 (7%), and 20 (6%).
▪ (↓) bwg through days 6-9 (125%) and 6-16 (29%).

<table>
<tr><th>Bw (g)</th><th>0 mg/kg bw</th><th>100 mg/kg bw</th><th>300 mg/kg bw</th><th>1000 mg/kg bw</th></tr>
<tr><td>Day 9</td><td>265</td><td>264</td><td>258</td><td>249**(↓6%)</td></tr>
<tr><td>Day 12</td><td>280</td><td>280</td><td>273</td><td>262**(↓6%)</td></tr>
<tr><td>Day 16</td><td>306</td><td>304</td><td>301</td><td>286**(↓7%)</td></tr>
<tr><td>Day 20</td><td>363</td><td>365</td><td>362</td><td>341**(↓6%)</td></tr>
</table>

<table>
<tr><th>Bw gain (g)</th><th>0 mg/kg bw</th><th>100 mg/kg bw</th><th>300 mg/kg bw</th><th>1000 mg/kg bw</th></tr>
<tr><td>Days (6-9)</td><td>8</td><td>8</td><td>4</td><td>-2** (↓125%)</td></tr>
<tr><td>Days (6-16)</td><td>49</td><td>48</td><td>47</td><td>35**(↓29%)</td></tr>
</table>

Food consumption:Food consumption:
▪ (↓) through days 6-9 (20%), 9-12 (13%), and (↑) through days 16-20 (13%, ndr).

<table>
<tr><th>Food consumption (g)</th><th>0 mg/kg bw</th><th>100 mg/kg bw</th><th>300 mg/kg bw</th><th>1000 mg/kg bw</th></tr>
<tr><td>Days (6-9)</td><td>82</td><td>79</td><td>76</td><td>66** (↓20%)</td></tr>
<tr><td>Days (9-12)</td><td>83</td><td>84</td><td>81</td><td>72** (↓13%)</td></tr>
<tr><td>Days (16-20)</td><td>84</td><td>89</td><td>93**(↑11%)</td><td>95**(↑13%)</td></tr>
</table>

Organ wt:
▪ (↑) rel liver wt (10%).
▪ (↓) abs gravid uterine wt (15%; ncdr).

<table>
<tr><th></th><th>0 mg/kg bw</th><th>100 mg/kg bw</th><th>300 mg/kg bw</th><th>1000 mg/kg bw</th></tr>
<tr><td>Gravid uterine weight (g)</td><td>75.3</td><td>75.7</td><td>76.0</td><td>64.2** (↓15%)</td></tr>
<tr><td>Corrected bw (g)</td><td>287.8</td><td>288.8</td><td>285.7</td><td>276.8</td></tr>
</table>
</td><td>Anonymous 27 (1996) (CA) B.6.6.2.3</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

Corrected bw change (g)	30.6	31.8	31.7	25.9

300 mg/kg bw/day

Food consumption:

- (↑) through days 6-9 (11%, ndr).

Organ wt:

- (↑) rel liver wt (8%).

Developmental toxicity

Findings	0 mg/kg bw	100 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
No. of females mated	24	24	24	24
No. of females pregnant	23	22	24	23
Abortions	0	0	0	0
No. of dead foetuses	0	0	0	1
Litters with viable foetuses	23	22	24	23
No. of corpora lutea	16.2	15.2	15.7	15.6
No. of implants	14.8	14.5	14.9	14.4
Litter size	13.7	13.7	14.2	13.2
Mean no. of males	7.2	5.9	7.0	7.3
Mean no. of females	6.6	7.8	7.2	5.8
Resorptions	25	19	17	27
Litters with resorptions	14 (60.9%)	13 (59.1%)	12 (50.0%)	11 (47.8%)
Mean foetal weight (g)	3.40	3.35	3.26	**2.83** (↓17%)
Female	3.33	3.28	3.19	**2.78** (↓16%)
Male	3.47	3.41	3.33	**2.90** (↓16%)

Historical Control Data from the conducting laboratory (1988-1992) in CD Rat provided in the original study

Parameter	Mean	Mean Range of study values		N
		Low	High	
Pregnancy rate	95.2	86.7	100	16
Mean/pregnant female				
Corpora lutea	16.7	14.9	18.2	16
Implants	15.5	13.7	16.9	16
Resorptions	0.9	0.5	1.6	16
Live fetuses	14.6	13.0	16.0	16
Mean fetal weight (g)				
Males	3.59			16
Females	3.40	3.19	3.59	16
Both sexes	3.50	3.28	3.69	16
Fetal sex ratio	1.0	0.9	1.3	16

Foetal weight

1000 mg/kg bw/day

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>▪ (↓) ♀/♂ (16%/16%) foetal weight.

300 mg/kg bw/day
▪ (↓) ♀/♂ (4%/4%; n.s) foetal weight.

Foetal malformations and variations

1000 mg/kg bw/day
External malformations
▪ 0.3% (1/304 foetuses) / 4.3% (1/23 litters) vs 0% in controls (0/316 foetuses / 0/23 litters) with edema.

Visceral malformations:
▪ 0.3% / 4.3% (1/304 foetuses / 1/23 litters) vs 0% in controls (0/316 foetuses / 0/23 litters) with abnormal course of the aortic arch.

Skeletal malformations
▪ 0.7% (1/146 foetuses) / 4.3% (1/23 litters) with fused ribs and misshapen thoracic centra vs 0% in controls (0/153 foetuses and 0/23 litters)
▪ 0.7% (1/146 foetuses) / 4.3% (1/23 litters) with misshapen lumbar and sacral vertebral centrum and absence of remaining sacral and caudal vertebrae vs 0.7% (1/143 foetuses) / 4.3% (1/23 litters) with absence of sacral and caudal vertebrae in controls.

Skeletal variations
▪ (↑) (79.5%) foetuses with at least one ossification variation/ retarded ossification (116/146 foetuses vs 62.1%, 95/153 in controls). Increment in litters was not significant (23/23 (100%) vs 21/23 (91.3%) in controls).

300 mg/kg bw/day

Visceral malformations
▪ 0.6% (1/175 foetuses examined vs 0/165 in controls; n.s.; ndr) with microphthalmia.

NOAEL _{developmental}: 300 mg/kg bw/day set in RAR (2016) based on decreased foetal bodyweight, and retardation in ossification at 1000 mg/kg bw/day. However, it has to noted that the decrease in foetal weights regarded of doubtful toxicological relevance due to the presence of maternal toxicity as it is further discussed in section 10.10.6.

NOAEL _{maternal toxicity}: 100 mg/kg bw/day based on increased maternal relative liver weight at 300 mg/kg bw/day.</td><td></td></tr>
<tr><td>Teratogenicity study in rabbits

Method: OECD TG 415 (1983) and US EPA 83-3.

GLP:Yes

Rabbit strain: New Zealand White

Sex: 17 mated females/group.

Deviations from current test guideline (OECD TG 414, 2018):</td><td>Test substance:
Flazasulfuron [SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 96.3% purity.

Oral gavage

0, 50, 150 and 450 mg/kg bw/day from day 6 to 18 of pregnancy both included</td><td>Maternal toxicity

Mortality: no mortality or morbidity signs related to treatment were detected in any of the study groups. One female in each control, 50 and 450 mg/kg bw/day dose group died due to a failure in the substance administration.

Clinical signs: An increased number of females with abortions was observed in 450 mg/kg bw/day dose group (5 dams) compared with other tested groups (1, 1 and 1 in control, 50 and 150 mg/kg bw/day dose groups, respectively).

Summary of clinical findings in females
<table><tr><th></th><th colspan="4">Groups (mg/kg bw/day)</th></tr><tr><th></th><th>0</th><th>50</th><th>150</th><th>450</th></tr><tr><td>No. animals examined</td><td>17</td><td>17</td><td>17</td><td>17</td></tr></table></td><td>Anonymous 28 (1988c) (CA) B.6.6.2.4</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

-The exposure period was from day 6 to 18 of gestation instead of the recommended period from implantation (e.g. day 5 post mating) to the day prior to scheduled caesarean section.

-Only 17 copulated females/group were used at the beginning of the study. The minimum required number is 20 female animals with implantation sites at necropsy.

-The following endpoints were not measured:

Number and percent of pre- and post-implantation losses; wt of the gravid uteri including cervix; anogenital distance.

Acceptable study

Parameters observed:

Maternal data:

Clinical signs, mortality, bw and bwg, food consumption.

Reproductive data:

Number (no.) of corpora lutea, no. implants, no. resorptions, placental wt, no. live and dead foetuses, no. dams with externally abnormal foetuses

Foetal data:

Foetus wt, foetus sex and foetus alterations (external, visceral and skeletal)

No. pregnant animals	16	15	15	14
non-pregnant animals	1	2	2	3
Clinical findings				
without abnormalities	12	12	13	7
Abortion	1	1	1	5
Found dead	1	1	0	1
Diarrhoea	4	3	1	2
Sedation/lateral position	1	0	0	0

Animals with eating disruption > 3 days

Dam	Days without food intake	Day of dead	Cause
0 mg/kg bw/day			
F4	12-17	18	Found dead
F5	22-26	27	Killed because abortion
F13	9-20	21	Killed in extremis
50 mg/kg bw/day			
F21	15-18	-	Not dead
F23	17-24	25	Killed because abortion
F24	17-25	26	Found dead
150 mg/kg bw/day			
F38	14-21	24	Killed because abortion
450 mg/kg bw/day			
F52	17-22	23	Killed because abortion
F56	12-19	20	Killed because abortion
F59	12-22	23	Killed because abortion
F60	12-22	23	Killed because abortion
F64	9-24	25	Found dead
F64	12.14	15	Found dead
F63	-	*23*	*Killed because abortion but without stopping food intake > 3 days*

It has to be noted that there was a non-pregnant animal (F61) which stopped eating on days 15-19 and survived the whole treatment. Abortion in F63 occurred without disruption of eating > 3 days though it was observed on days 6-7 of treatment.

Bodyweight and bodyweight gain:

- N.S. differences in any dose group compared with control group.

450 mg/kg bw/day

Food consumption:

- N.S. differences in any dose group compared with control group.
- (↑) number of animals stopped eating >3 days after initiation of treatment (43%, 6/14 animals, n.s vs 19%, 3/16 animals in controls).

Developmental toxicity

- N.S. differences in reproductive data and foetal weights in any dose group compared with controls. The only effect was the increased number of abortions at 450 mg/kg bw/day (5 dams) compared to the other dose groups (1, 1 and 1 in control, 50 and 150 mg/kg bw/day, respectively). All abortions with the exception of one occurring at the top dose level were seen in animals which stopped eating > than 3 days.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

Findings	0 mg/kg bw	100 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
No. of dams with foetuses	13	13	14	8
No. of corpora lutea	10.5	8.7	9.9	10.3
No. of implants	9.2	7.2	9.4	8.5
Abortions	1	1	1	5
Resorptions Early Late Total	 18 1 19	 7 6 13	 8 1 9	 11 4 15
Foetal mortality (early and late resorptions)	17.4%	17.8%	6.0%	19.2%
Total number of live foetuses per litter	7.8 (101)	6.2 (81)	8.8 (123)	6.6 (53)
Mean foetal weight				
Female (g)	35.3	36.6	38.2	39.4
Male (g)	36.2	36.8	38.4	38.0

External alterations/skeletal and visceral malformations

- No adverse effect of treatment was indicated from external, skeletal and visceral malformations.

	0 mg/kg bw	100 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
External malformations [no. foetuses (no. litters)]				
Gastroschisis	1 (1)	0	0	0
Acephaly	1 (1)	0	0	0
Club hand	1 (1)	0	0	0
Brachyury	2 (2)	0	0	0
Total foetuses with malformations (no. litters)	3 (2)	0	0	0
Skeletal malformations [no. foetuses (no. litters)]				
Absence of cranial bones (acephaly)	1 (1)	0	0	0
Fusion of two or three ossification centers	0	2 (1)	4 (2)	0
Fusion of caudal vertebrae	1 (1)	0	0	0
Fusion of two ribs	1 (1)	0	0	0
Total foetuses with malformations (no. litters)	3 (2)	2 (1)	4 (2)	0
Skeletal variations [no. foetuses (no. litters)]				
Asymmetric sternebrae	1 (1)	0	0	2 (1)
Splitting or smallness of the thoracic central body	1 (1)	0	0	0
Total foetuses with variations (no. litters)	2 (2)	0	0	2 (1)
Visceral malformations [no. foetuses (no. litters)]				
Agenesis of the pulmonary trunk	0	0	0	1 (1)
Total foetuses with variations (no. litters)	0	0	0	1 (1)

NOAEL $_{\text{developmental}}$ **>450 mg/kg bw/day*** due to no developmental effects were detected at any dose level.
NOAEL $_{\text{maternal toxicity}}$**: 150 mg/kg bw/day** based on higher incidence

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		of abortions and a higher number of females that stopped eating for more than 3 days after initiation of administration at 450 mg/kg bw/day. **NOAEL$_{developmental}$ in RAR 2016 was established in 150 mg/kg bw/day based on increased number of abortions in 450 mg/kg bw/day dose group. However, the MSCA deems that most abortions occurred after these dams stopped eating for more than 3 days, and taken together, these effects are consequence of maternal toxicity triggered to repeated test substance administration at the high dose level. In addition, all the abortions occurred in the last third of the pregnancy, specifically, through pregnancy days 20-23, and this period is out of the organogenesis in rabbits (pregnancy days 6-18). On the other hand no adverse effects were detected in pups in the same dose level.*	

Table 73: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 74: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Historical control data on spontaneous foetal anomalies in the rat. *J. Animal Reproduction, 29 (suppl. 22):pp 31-71 and 1983-1986* Method: Not applicable **Supportive information** Wistar Imamichi HCD comes from the same laboratory that performed the OECD TG 414 studies in this strain of rat. Sprague Dawley HCD does not come from the laboratory that performed the OECD TG 414 study in this strain of rat.	None. Report on control group animals	Rat strain: Wistar-Imamichi (WI) and Sprague-Dawley (SD) rats strains Period of study: 1975-1982 *Reproductive data:* Number (no.) of dams, no. implantations, prenatal mortality, no. live foetuses. *Foetal data:* Mean foetal wt, spontaneous external malformations, spontaneous skeletal malformations and variations and spontaneous internal	***Foetal data*** *Spontaneous visceral alterations* ▪ *VSD*: 1.47% (range 0-11.3%) in WI rats and 0.3% (range 0-1.8%) in SD rats. ▪ *Renal pelvis dilation*: 2% (0-5.6%) in WI and 8.2% (2.5-13.5%) in SD rats. ▪ *Hydroureter*: 6.1% (0-25.2%) in WI and 19.7% (0-54.5%) in SD rats. **Spontaneous visceral incidences** **Strain** / **Wistar-Imamichi** / **Sprague-Dawley** **No. of dams examined**: 594 / 166 **No. of foetuses examined**: 3809 / 991 **Ventricular septal defect** No. foetuses: 56 / 3 mean (%): 1.47 / 0.3 range (%): 0-11.3 / 0-1.8 **Dilation of renal pelvis** No. foetuses: 79 / 82 mean (%): 2 / 8.2 range (%): 0-5.6 / 2.5-13.5 **Hydroureter** No. foetuses: 233 / 196 mean (%): 6.1 / 19.7 range (%): 0-25.2 / 0-54.5	Anonymous 29 (1983)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		malformations		
Temporal changes in incidence of ventricular septal defect in Wistar-Imamichi foetal and breast-fed rats *Annual Report of the Imamichi Institute for Animal Reproduction, 1984* Method: Not applicable. **Supportive information** These historical control data (HCD) come from the same laboratory that performed the rat OECD TG 414 studies	None.	Rat strain: Wistar-Imamichi -Original conducted assay in breast-fed rats (no. of animals not detailed). -Retrospective investigation (1981-1984 period): 8 studies/20 rats per study *Foetal data:* -Incidence of VSD in pups from 20 days of gestation to 14 days of postnatal age in original assay. -Incidence of VSD at 21 days of gestation in the retrospective study.	***Foetal data (Data summary from previous studies)*** *Visceral alterations* ▪ *Ventricular septal defect (VSD)*: 3,01% (36/1196 foetuses examined). ▪ *Renal pelvis dilation*: 1.35% (33/2444 foetuses examined) ▪ *Hydroureter*: 5.48% (134/2444 foetuses examined). ***Ventricular septal defect incidences*** *Breast-fed rats* ▪ 7.6 % at 20 days of gestation. ▪ 3.01 % at 21 days of gestation. ▪ 0 % at ≥1 day of postnatal age. *Retrospective study* ▪ 2.9% at 21 days of gestation.	Anonymous 30 (1984)
The effects of ephedrine on the foetal rat heart *J. of Tokyo Women's Medical College, 57 (5), 347-357* Method: Not available. **Supportive information** considered relevant for the assessment of the concerning malformation VSD	Test substance: **Ephedrine** [(1R,2S)-2-(methylamino)-1-phenylpropan-1-ol]	Rat strain: Wistar-Imamichi rats Single intraperitoneal injection Dose: 0, 0.1, 1, 10 and 50 mg/kg bw/day on day 9, 10 or 11 of pregnancy. The specific day of the injection is not available. *Reproductive data:* Number (no.) of litters, no. implants, no. resorptions, no. live and dead foetuses	***Developmental toxicity*** ***Foetal data*** **50 mg/kg bw/day** *Cardiac alterations:* ▪ (↑) foetuses with ventricular septal defect (VSD) (27.2%, 46/169 foetuses examined vs 6/188 in controls). **10 mg/kg bw/day** *Cardiac alterations:* ▪ (↑) foetuses with VSD (21.7%, 40/184 foetuses examined). **1 mg/kg bw/day** *Cardiac alterations:* ▪ (↑) foetuses with VSD (15.7%, 13/83 foetuses examined). **0.1 mg/kg bw/day** *Cardiac alterations:* ▪ (↑) foetuses with VSD (8.2%, 6/73 foetuses examined). **Cardiac malformation induced by ephedrine in rat foetuses (20 days of gestation)**	Anonymous 31 (1987)

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr>
<td></td>
<td></td>
<td>Foetal data: Foetus sex, Foetus wt, foetal crown-rump length and foetus alterations (cardiac malformations).</td>
<td>
<table>
<tr><th>Dose</th><th>No. litters</th><th>live foetuses (per litter)</th><th>VSD(%)</th></tr>
<tr><td>Control</td><td></td><td></td><td></td></tr>
<tr><td>distilled water</td><td>5</td><td>62 (12.4)</td><td>2/62 (3.2)</td></tr>
<tr><td>untreated</td><td>10</td><td>126 (12.6)</td><td>4/126 (3.2)</td></tr>
<tr><td>0.1</td><td>5</td><td>73 (14.6)</td><td>6/73 (8.2)</td></tr>
<tr><td>1</td><td>6</td><td>83 (13.8)</td><td>13/83 (15.7)*</td></tr>
<tr><td>10</td><td>15</td><td>184 (12.3)</td><td>40/184 (21.7)*</td></tr>
<tr><td>50</td><td>13</td><td>169 (13.0)</td><td>46/169 (27.2)*</td></tr>
</table>
*statistically significant p<0.05
</td>
<td></td>
</tr>
<tr>
<td>Spontaneous and induced alterations in the cardiac membranous ventricular septum in rats.

Teratology, 55 (3), 185-94

Method: Not applicable.

Supportive information considered relevant for the assessment of the concerning malformation VSD</td>
<td>The objective of the study was to describe specific alterations of the cardiac membranous ventricular septum of foetal, weanling and adult Sprague dawley rats.

Positive controls:
Trimethadione [3,5,5-trimethyl-1,3-oxazolidine-2,4-dione]

Trypan blue [5-amino-3-[(E)-2-{4'-[(E)-2-(8-amino-1-hydroxy-3,6-disulfonaphthalen- 2-yl)diazen-1-yl]-3,3'-dimethyl-[1,1'-biphenyl]-4-yl}diazen-1-yl]-4-hydroxynaph</td>
<td>Rat strain: Sprague-Dawley

Treatments/ groups:

Spontaneous lesion group: animals not treated with any substance.

Positive control agents:

Trypan-blue (subcutaneous injection): 50 mg/kg bw/day on day 7 to 9 of pregnancy. 16 mated females.

Trimethadione: (Oral gavage): 400 mg/kg bw/day on day 9 and 10 of pregnancy. 5 mated females.

Other groups:

Ad libitum: 9 mated females,

Diet-restriction: 7 g food/day/ female (↓25% compared with control group). 10 mated females</td>
<td>
Developmental toxicity

Visceral alterations
Foetal data (21 day postcoitus)
Spontaneous lesions group
▪ NPD: 38.1% at day 17, 10.5% at day 19 and 4.3% at day 21.
▪ mVSD: 0% at day 17, 0% at day 19 and 2% at day 21.
Trimethadione (positive control)
▪ NPD: 0%.
▪ mVSD: 93%.
Trypan blue group (positive control)
▪ NPD: 9.9%.
▪ mVSD: 9.2%.
Ad libitum
▪ NPD: 1.7%.
▪ mVSD: 1.6%.
Diet-restricted
▪ NPD: 3.5%.
▪ mVSD: 1.3%.

Pups data (21 day postnatal)
Weanling
▪ NPD: 11.8%.
▪ mVSD: 0%.

Adult data (5-10 months old)
▪ NPD: 9.1%.
▪ mVSD: 0%.

Incidence of membranous ventricular septal observations detected by fresh visceral examinations in spontaneous lesion group
<table>
<tr><th></th><th colspan="3">Foetus</th><th>Weanling</th><th>Adult</th></tr>
<tr><th>Malformations</th><th>Day 17 pc</th><th>Day 19 pc</th><th>Day 21 pc</th><th>day 21 pn</th><th>5-10 months old</th></tr>
<tr><td>NPD</td><td colspan="5"></td></tr>
<tr><td>mean±SEM</td><td>38.1±3.65</td><td>10.5±2.66</td><td>4.3±1.16</td><td>11.8±4.13</td><td>9.1</td></tr>
<tr><td>No. foetuses affected/ no. foetuses examined</td><td>41/106</td><td>9/80</td><td>25/550</td><td>18/188</td><td>7/77</td></tr>
</table>
</td>
<td>Anonymous 32 (1997)</td>
</tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr>
<td></td>
<td>thalene-2,7-disulfonic acid]</td>
<td>Foetal data:
-Incidence of spontaneous non-patent depressions (NPD) and membranous ventricular septal defect (mVSD) observed in the spontaneous lesion group on gestation day 17, 19 and 21; 21 postnatal day (weanling) and adult (5-10 months old). For positive controls, ad libitum and diet restricted groups observations were made on postcoital day 21.

Incidence of skeletal observations in ad libitum and diet restricted litters (21 days of pregnancy).</td>
<td>
<table>
<tr><td>No. litters affected/ no. litters examined</td><td>7/7</td><td>5/6</td><td>15/40</td><td>8/14</td><td>-</td></tr>
<tr><td>mVSD</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>mean±SEM</td><td>0</td><td>0</td><td>2±0.66</td><td>0</td><td>0</td></tr>
<tr><td>No. animals affected/ no. animal examined</td><td>0/106</td><td>0/80</td><td>13/550</td><td>0/188</td><td>0/77</td></tr>
<tr><td>No. litters affected/ no. litters examined</td><td>0/7</td><td>0/6</td><td>9/40</td><td>0/14</td><td>-</td></tr>
</table>
NPD: Non-patent depressions
mVSD: membranous ventricular septal defect
pc: postcoitus
pn:postnatal.
Means represents ratio between no. foetuses affected/no. foetuses examined

Incidence of foetal heart and skeletal observations in ad libitum and diet restricted litters.
<table>
<tr><th>Observations</th><th>Ad libitum</th><th>Diet-restricted</th></tr>
<tr><td>Heart</td><td></td><td></td></tr>
<tr><td>Non-patent depressions
mean±SEM</td><td>1.7±1.13
(2/122)
[2/9]</td><td>3.5±2.09
(3/115)
[3/10]</td></tr>
<tr><td>Ventricular septal defect
mean±SEM</td><td>1.6±1.09
(2/122)
[2/9]</td><td>1.3±1.25
(3/115)
[1/10]</td></tr>
<tr><td>Skeleton</td><td></td><td></td></tr>
<tr><td>Sternebrae (i.o.)
mean±SEM</td><td>0
(0/60)
[0/9]</td><td>30.12±7.50
(16/56)
[7/10]</td></tr>
<tr><td>Sternebrae (u.)
mean±SEM</td><td>0
(0/60)
[0/9]</td><td>24.76±11.51
(17/56)
[4/10]</td></tr>
<tr><td>Forepaw, metacarpals, (u.)
mean±SEM</td><td>0
(0/60)
[0/9]</td><td>15.36±6.74
(9/56)
[4/10]</td></tr>
<tr><td>Forepaw, phalanges, (i.o.)
mean±SEM</td><td>0
(0/60)
[0/9]</td><td>11.01±5.54
(4/56)
[4/10]</td></tr>
<tr><td>Hindpaw, phalanges, (i.o.)
mean±SEM</td><td>0
(0/60)
[0/9]</td><td>37.56±9.78
(22/56)
[8/10]</td></tr>
</table>
i.o.: incomplete ossification
u: unossified
(): no. foetuses affected/no. foetuses examined
[]: no. litters affected/no. litters examined
Means represents ratio between no. foetuses affected/no. foetuses examined.
</td>
<td></td>
</tr>
<tr>
<td>Ventricular septal defects in WKY rats

Hypertension, 40 (2), 175-178

Method: Not applicable.

Supportive information considered relevant for the</td>
<td>None. Control animals.</td>
<td>Rat strain:
Wistar-Kyoto (WKY rats)

Adult data:
Echocardiogra_phic examination, hemodynamic examination,</td>
<td>Adult data
Visceral alterations
▪ VSD: 27% (13/48 animals; 10♂ and 3♀) in Charles River WKY rats.
▪ VSD: 10% (2/20 animals; 2♂) in Harlan WKY rats.
▪ (↑) 180% of right ventricle wt in abnormal rats compared with normal rats.
▪ (↑) 8% (n.s.) of left ventricle wt in abnormal rats compared with normal rats.</td>
<td>Anonymous 33 (2002)</td>
</tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td>assessment of the concerning malformation VSD</td><td></td><td>pathologic study of the heart, and left and right ventricles wt.</td><td>▪ (↑) 44% of biventricle wt in abnormal rats compared with normal rats.
Echocardiographic findings
<table>
<tr><th>Company</th><th>no.</th><th>sex</th><th>Age (wk)</th><th>VSD +PR</th><th>VSD + PR + other</th><th>Other defects</th><th>Abnormal (%)</th></tr>
<tr><td>Charles River</td><td>20</td><td>♂</td><td>12</td><td>5</td><td>3</td><td>0</td><td>8 (40)</td></tr>
<tr><td>Charles River</td><td>20</td><td>♀</td><td>12</td><td>2</td><td>1</td><td>2 (PS)</td><td>5 (25)</td></tr>
<tr><td>Harlan</td><td>20</td><td>♂</td><td>12</td><td>2</td><td>0</td><td>1 (PDA)</td><td>3 (15)</td></tr>
<tr><td>Charles River*</td><td>8</td><td>♂</td><td>52</td><td>0</td><td>2</td><td>1 (AR)</td><td>3 (15)</td></tr>
<tr><td>Total (%)</td><td>68</td><td></td><td></td><td>9 (12)</td><td>6 (9)</td><td>4 (7)</td><td>19 (28)</td></tr>
</table>
VSD: Ventricular septal defect
PR: pulmonary regurgitation
PS: pulmonary stenosis
PDA: patent ductus arteriosus
AR: aortic regurgitation
* one-year-old WKY rats already present in the laboratory</td><td></td></tr>
<tr><td>Cohort study of ventricular septal defects in humans.
Cardiology Young, 12 (4), 357-36
Method: Not applicable.
Supportive information on ventricular septal defect</td><td>None, prospective cohort study performed in humans</td><td>290 patients
Analysis of the size and morphology of VSD.</td><td>Summary data of the cohort study
<u>290 children</u>
Size of the VSD:
▪ Small: 227 cases (78%)
-Muscular: 167 cases: 69% closed spontaneously (115/167).
-Perimembranous: 59 cases: 47% closed spontaneously (28/59).
- 1 position none stated: closed spontaneously.
▪ Moderate: 43 cases (15%)
-Muscular: 10 cases: 80% closed spontaneously (8/10).
-Perimembranous: 33 cases: 9% closed spontaneously (3/33).
▪ Large: 20 cases (7%)
-Muscular: 3 cases: 0% closed spontaneously.
-Perimembranous: 15 cases: 0% closed spontaneously.
-1 doubly committed.
-1 both muscular and perimembranous.</td><td>Turner, S.W. et al (2002)</td></tr>
<tr><td>Postnatal closure of membranous ventricular septal defects in rats
Birth Defects Res. B. Dev. Reprod. Toxicol. 71 (3), 185-189
Method: Not applicable.
Supportive information on ventricular septal defect</td><td>Test substance: Trimethadione [3,5,5-trimethyl-1,3-oxazolidine-2,4-dione]</td><td>Rat strain: Sprague–Dawley rats [Crl:CDs (SD)IGS BR]
Oral gavage
0, 400 and 600 mg/kg bw/day on gestation day 9 and 10.
48 mated females/ control group</td><td><u>Maternal toxicity</u>
Mortality: no mortality or morbidity signs were observed in females at any dose level.
Clinical signs: No treatment-related clinical signs were observed.
Bodyweight: No effects on mean bw were observed throughout study.
<u>Developmental toxicity</u>
Reproductive data
600 mg/kg bw/day
▪ (↑) postimplantation loss (7.2% vs 2.9% in controls).
▪ (↑) early resorptions (4.7% vs 2.4% in controls; n.s.).
▪ (↑) late resorptions (2.1% vs 0.6% in controls; n.s.).
▪ (↑) death foetuses (0.4% vs 0% in controls; n.s.).
▪ (↓) foetal wt (7%; n.s.).</td><td>Anonymous 34 (2004)</td></tr>
</table>

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td>40 mated females/ trimethadione group

Maternal data:

Clinical signs, mortality, bw.

Reproductive data:

Number (no.) of corpora lutea, no. implants, no. resorptions (early and late), no. live and dead foetuses, postimplantation losses.

Foetal data:

Foetus wt, foetus sex and foetus alterations (external, visceral and skeletal).</td><td>▪ (↓) wt of foetuses with VSD and vessel anomalies (22%).

400 mg/kg bw/day
▪ (↑) postimplantation loss (4.3% vs 2.9% in controls; n.s.).
▪ (↑) death foetuses (1.2% vs 0% in controls; n.s.).

Foetal data (gestation day 21)

600 mg/kg bw/day
Visceral examination
▪ (↑) all VSD incidences (49.8% vs 0.6% in controls).
▪ (↑) onlyVSD incidences (39.6% vs 0.6% in controls).
▪ (↑) VSD and vessel anomalies (10.1% vs 0% in controls).

400 mg/kg bw/day
Visceral examination
▪ (↑) all VSD incidences (7.6% vs 0.6% in controls).
▪ (↑) onlyVSD incidences (7.6% vs 0.6% in controls).

Pups data

600 mg/kg bw/day
▪ (↓) pups survival through postnatal days 0-1 (20%).
▪ (↓) pups survival through postnatal days 1-4 (14%).
▪ (↑) pup mortality from birth to postnatal day 21 (27%; 65/242 animals vs 3%; 9/320 animals in controls, n.s.).

Visceral examination
▪ (↑) only VSD incidences (6.4%; 14/176 animals vs 0.03%; 1/311 animals in controls) at postnatal day 21.

Incidence of isolated VSD (per litter)

<table>
<tr><th></th><th colspan="3">GD 21 (%)</th><th>PND 21 (%)</th></tr>
<tr><th></th><th>All VSD</th><th>Only VSD</th><th>VSD and vessel anomalies</th><th>Only VSD</th></tr>
<tr><td>Control</td><td>0.6±2.13
(2/338)</td><td>0.6±2.13
(2/338)</td><td>0.0±0
0/338</td><td>0.03±1.45
(1/311)</td></tr>
<tr><td>400 mg/ mg bw/ day</td><td>7.6±11.41*
(28/268)</td><td>7.6±11.41*
(28/268)</td><td>0.0±0
0/268</td><td>0±0
(0/258)</td></tr>
<tr><td>600 mg/ mg bw/ day</td><td>49.8±29.58*
(128/264)</td><td>39.6±25.3*
(106/264)</td><td>10.1±17.98*
(22/264)</td><td>6.4±8.22*
(14/176)</td></tr>
</table>
GD: gestation day
PND: Postnatal day
Data presented as the mean±SD of the % per litter occurrence.
(Affected/examined is total number of offspring affected/Total number of offspring examined).
*Statistically significant p<0.01</td><td></td></tr>
<tr><td>Review of ventricular septal defects in rats.

Unpublished report</td><td>Review</td><td></td><td><u>Developmental toxicity study with flazasulfuron in Wistar-Imamichi rats (Anonymous 26, 1988b)</u>

▪ (↑) VSD at 1000 mg/ kg bw/day (6.6%).
▪ (↑) VSD at 300 mg/ kg bw/day (4%).

Summary of ventricular septal defects</td><td>Anonymous 35 (2019)</td></tr>
</table>

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Finding	Dose Group (mg/kg bw/day)				Historical control		
	0	100	300	1000	1975-82	1983-86	All data
Total number of fetuses examined	156	153	149	122	3809	2757	6566
Total number of litters	23	23	23	20	NA	NA	NA
Incidence of dams with a pup with VSD	1	1	6	7	NA	NA	NA
Incidence of pups with VSD (N)	2	1	6	8	56	42	98
% incidence of pups with VSD	1.3%	0.7%	4.0%	6.6%	1.47% 0-11.3%	1.52% 0-5.3%	1.49% 0-11.3%

NA= Not available

Developmental toxicity of flazasulfuron in Sprague-Dawley rats (Anonymous 27, 1996)

- There was no evidence of VSD at any dose level.

Developmental toxicity of flazasulfuron in rabbits (Anonymous 28, 1988c)

- There was no evidence of VSD at any dose level.

Analysis of the background incidence of VSD in Wistar-Imamichi rats (Anonymus 30, 1984)

Analysis of studies for malformations

2,444 samples of fetal rats delivered from 361 dams	
Finding	Fetuses (% Incidence)
Subcutaneous edema	11 (0.45%)
Anophthalmia	1 (0.04%)
Thymic hypoplasia	1 (0.04%)
Testicular hypoplasia	1 (0.04%)
Angiectopia of the heart and great vessel	1 (0.04%)
Bicuspid valve hypoplasia	3 (0.12%)
Tricuspid valve hypoplasia	3 (0.12%)
Abnormal appearance of the heart	2 (0.08%)
Total	**23 (0.94%)**
Ventricular septal defect	36 (3.01%)
Renal pelvis dilatation	33 (1.35%)
Hydroureter	134 (5.48%)

Incidence of ventral sepal defect in other common rat strains

- (↑) 20-100 fold higher VSD incidence in Wistar-Imamichi rats compared with other rat strains.

Summary of historical control data from Charles River laboratories

Strain	Source	Litter/fetal incidence	Range/study		
			Average %	N	%
Wistar [Crl:WI(Han)]	Studies 2003-16	Litter incidence	0.55	0-1	0-5.0
		Fetal incidence	0.08	0-1	0-1.0
Crl:CD BR	MARTA 1993	Litter incidence	0.134	Not reported	0-8.33
		Fetal incidence	0.016	Not reported	0-0.85
	Studies 2011-15	Litter incidence	0.21	0-1	0-4.3
		Fetal incidence	0.03	0-1	0-0.6
Data from the Imamichi Institute for the Wistar Imamichi rat for comparison					
Wistar-Imamichi rat	Studies 1975-82	Litter incidence	NA	NA	NA
		Fetal incidence	1.47	NA	0-11.3
	Studies 1983-86	Litter incidence	NA	NA	NA
		Fetal incidence	1.52	NA	0-5.3

NA= Not available

VSD reversibility (Anonymous 30, 1984)

- 7.6% of VSD at gestation day 20.
- 3.01% of VSD at gestation day 21.
- 0% of VSD at postnatal day 1 or older.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of flazasulfuron (SL-160 technical) was investigated in four prenatal developmental toxicity studies, three in rats (B.6.6.2.1, B.6.6.2.2 and B.6.6.2.3) and one in rabbit (B.6.6.2.4). All studies predate the current OECD Test Guideline Number 414 (2018) and do not include the recommended extended dosing period (i.e. from implantation to one day prior to the day of scheduled kill). However, these studies are considered adequate and relevant for evaluation of the potential of flazasulfuron to induce developmental effects.

The preliminary teratogenicity study in rats (Anonymous 25, 1988a; B.6.6.2.1) was designed to select the appropriate dose levels for the main teratogenicity study (B.6.6.2.2). In this pilot study, flazasulfuron was tested at dose levels of 0, 50, 200, 500 and 1000 mg/kg bw/day, in which 8 copulated females were assigned to each test group.

Neither maternal mortality or morbidity signs, nor treatment related clinical signs were observed at any dose level.

Female bodyweights were decreased in a statistically significant manner in the high dose dams at gestation days 9 (5%), 12 (6%) and 18 (6%) compared with control group. Besides, reduced bodyweight gain was detected in the same group during organogenesis (145%, days 6-9). Food consumption was also significantly decreased on days 6-9 (37%) and 9-12 (30%). At 500 mg/kg bw/day dose group, a statistically significant decrease of bodyweight gain was also observed through days 6-9 (64%).

Table 75: Female (Maternal) Body Weights (g)

Groups					
Pregnancy (days)	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**500 mg/kg bw**	**1000 mg/kg bw**
0	246±9(8)	246±8(8)	246±8(7)	246±8(8)	243±10(8)[a]
6	275±8(8)	277±8(8)	280±14(7)	278±9(8)	276±8(8)
9	286±8(8)	289±9(8)	288±14(7)	281±9(8)	271±16(8)*
12	305±10(8)	307±7(8)	306±16(7)	300±14(8)	287±20(8)*
15	322±9(8)	329±8(8)	327±15(7)	322±17(8)	306±18(8)
18	365±10(8)	371±12(8)	371±16(7)	366±20(8)	342±25(8)*
21	404±15(8)	408±16(8)	412±20(7)	399±25(8)	369±42(8)

( ) = number of animals
* = statistically significant p = < 0.05

Table 76: Female (Maternal) Body Weight Gain (g)

Groups					
Pregnancy (days)	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**500 mg/kg bw**	**1000 mg/kg bw**

0-6	29±3(8)	31±2(8)	34±6(7)	32±7(8)	33±5(8)
6-9	11±5(8)	13±4(8)	8±2(7)	4±3(8)*	-5±14(8)**
9-12	19±2(8)	18±3(8)	18±3(7)	18±5(8)	15±6(8)
12-15	18±3(8)	22±5(8)	21±2(7)	22±5(8)	19±7(8)
15-18	42±9(8)	42±8(8)	45±4(7)	44±7(8)	37±7(8)
18-21	39±6(8)	37±8(8)	41±5(7)	34±7(8)	27±18(8)*

( ) = number of animals
* = statistically significant p = < 0.05
**** = statistically significant p = < 0.01**

Table 77: Female (Maternal) Food Consumption

Groups					
Pregnancy (days)	**0 mg/kg bw**	**50 mg/kg bw**	**200 mg/kg bw**	**500 mg/kg bw**	**1000 mg/kg bw**
0-6	21.7±1.7(8)	22.2±1.0(8)	22.3±2.0(7)	22.3±2.4(8)	22.1±1.4(8)
6-9	24.1±1.8(8)	24.4±2.2(8)	23.4±3.2(7)	19.9±2.8(8)	15.3±6.3(8)**
9-12	27.7±2.8(8)	27.7±1.8(8)	27.7±1.3(7)	24.6±3.3(8)	19.3±4.1(8)**
12-15	26.1±2.6(8)	28.1±1.1(8)	27.3±2(7)	26.0±3.4(8)	22.0±1.3(8)
15-18	30.1±2.0(8)	31.5±2.5(8)	31.3±2.3(7)	30.7±3.7(8)	28.4±2.0(8)
18-21	26.2±2.0(8)	27.9±3.2(8)	29.9±2.4(7)	28.7±3.8(8)	28.6±3.3(8)

( ) = number of animals
** = statistically significant p = < 0.01

Regarding data on developmental toxicity, mean foetal bodyweight was statistically significantly lower at the highest dose level, compared with control group (4% and 6% for females and males, respectively). However, the effect is not considered relevant for development regarding the absence of a clear dose-dependancy and the low magnitude of the reduction

Foetal mortality was 19.4% at the top dose as compared to 4.8% in control, and mean number of live foetuses per dam was 11.6 at the top dose as compared to 14.9 in control. These effects on pup survival can be treatment related considering the magnitude of the increase but a potential linked to marked maternal toxicity manifested by reduction in bodyweight, bodyweight gain and food consumption cannot be ruled out.

External examination revealed low incidences of malformations which were considered to occur spontaneously. This observation mainly consisted of associated malformations such as preaxial polydactyly in the hind limbs in one 1000 mg/kg bw/day foetus; vestigial tail and anal atresia in one 500 mg/kg bw/day foetus; and edema, micrognathia, cleft palate and preaxial polydactyly in the hind limbs in another foetus in the 50 mg/kg bw/day group.

Skeletal and visceral observations were only performed on the 0, 50 and 1000 mg/kg bw/day dose groups in approximately half of the fetuses. The occurrence of the effects is regarded incidental since they are non-significant and isolated. The only concerning effect is the increase of ventricular septal defect (VSD) observed at the highest dose level. This effect has been observed in other rat studies and it is not regarded relevant for classification as further discussed below.

Skeletal variations such as supernumerary 14^{th} ribs, were detected in two foetuses (4%) from the same litter (dam no. 40) at 1000 mg/kg bw/day. At 50 mg/kg bw/day in the foetus exhibiting external malformations it was also observed the following skeletal malformations: splitting of 1^{st} and 2^{nd} sternebrae and fusion of 3^{rd}-$4t^{h}$-$5t^{h}$ sternebrae.

Regarding visceral examinations, an increase incidence of foetuses with ventricular septal defect (VSD) was detected in the high dose group (6.8%). They were observed in two foetuses from the same litter of the dam no. 37, and in one foetus from dam no. 39. However, VSD was also observed in one foetus from the dam no.7

of the control group (1.7%). No VSD events were detected at 50 mg/kg bw/day dose group. If we compared these values with the two sets of historical control data (HCD) from the same conducting laboratory (1975-1982 and 1983-1986, respectively), the VSD observed at 1000 mg/kg bw/day group was slightly higher than 1983-1986 HCD (range 0-5.3%) and within of 1975-1982 HCD (range 0-11.3%)[4].

At the lowest dose level, visceral analysis revealed one foetus from dam no. 10 with dilation of renal pelvis and ureter not related to test substance administration since it was not dose-related and presented low incidence.

Based on these results, definitive dose levels of 0, 100, 300 and 1000 mg/kg bw/day were established for the main teratogenicity study in Wistar-Imamichi rats.

The main teratogenicity study in rats (Anonymous 26, 1988b; B.6.6.2.2) was designed to evaluate the potential teratogenicity effect of flazasulfuron (SL-160 technical) in Wistar-Imamichi rats. Flazasulfuron was tested at dose levels of 0, 100, 300, and 1000 mg/kg bw/day, to each of which 23 copulated females were assigned dose level based on results of preliminary study (B.6.6.2.1) summarised above.

Neither maternal mortality or morbidity signs, nor treatment related clinical signs were observed at any dose level.

In the 1000 mg/kg bw/day group, dams bodyweights were statistically significantly lower from gestation day 9. A reduced bodyweight gain was observed during organogenesis (days 6-9) at mid and high dose groups (50% and 150%, respectively). Moreover, 1000 mg/kg bw/day dams exhibited reduced bodyweight gain through 12-15 days (21%).

Table 78: Female (Maternal) Body Weights (g)

Pregnancy (days)	**0 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**1000 mg/kg bw**
0	236±9(23)	236±8(23)	236±7(23)	236±6(22)[a]
6	268±9(23)	269±10(23)	270±9(23)	268±9(22)[a]
9	280±11(23)	277±10(23)	276±11(23)	262±15(22)**
12	300±13(23)	298±11(23)	295±12(23)	281±21(22)**
15	324±12(23)	318±11(23)	316±13(23)	300±26(22)**
18	363±14(23)	357±13(23)	357±16(23)	337±30(22)**
21	400±17(23)	394±15(23)	396±18(23)	370±41(22)**

( ) = number of animals
** = statistically significant p = < 0.01

Table 79: Female (Maternal) Body Weight Gain (g)

Pregnancy (days)	**0 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**1000 mg/kg bw**
0-6	32±5(23)	32±5(23)	34±5(23)	33±7(22)[a]
6-9	12±4(23)	9±4(23)	6±4(23)**	-6±11(22)**
9-12	20±5(23)	20±4(23)	19±4(23)	19±11(22)
12-15	24±4(23)	21±4(23)	21±5(23)	19±6(22)**
15-18	39±5(23)	39±6(23)	41±6(23)	37±8(22)
18-21	37±10(23)	37±6(23)	40±4(23)	33±13(22)**

( ) = number of animals
** = statistically significant p = < 0.01

[4] Anonymous 29 (1983)

Food consumption was statistically significantly lower through days 6-9 (11%) and 6-15 (20-36%) at mid and high dose groups, respectively.

Table 80: Female (Maternal) Food Consumption

Pregnancy (days)	**0 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**1000 mg/kg bw**
0-6	22.7±2.3(23)	21.3±1.9(23)	22.2±2.4(23)	21.8±1.8(22)
6-9	23.6±2.3(23)	23.2±2.6(23)	21.1±2.4(23)*	15.2±4.9(22)**
9-12	26.1±2.7(23)	25.6±1.8(23)	24.4±2.5(23)	20.1±5.1(22)**
12-15	29.2±4.8(23)	28.2±1.8(23)	27.6±2.1(23)	23.3±4.5(22)**
15-18	30.0±3.5(23)	29.6±2.1(23)	30.3±2.3(23)	28.5±2.5(22)
18-21	24.9±3.8(23)	25.3±2.5(23)	26.8±2.6(23)	28.1±2.2(22)**

( ) = number of animals
* = statistically significant p = < 0.05
** = statistically significant p = < 0.01

These findings show maternal toxicity at 1000 mg/kg bw/day dose level, and to a lesser extent, but also clearly, at 300 mg/ kg bw/day dose group during organogenesis. The severity of maternal toxicity at this top dose level is not sufficient to be considered a direct cause of the following developmental effects, with the exception of reduction of foetal weights.

As in the pilot study, at 1000 mg/kg bw/day, an increase in the foetal incidences of mortality was detected (20.8% at 1000 mg/kg bw/ vs 5.5%, 3.7% and 4.7% in controls, 100 and 300 mg/kg bw/day groups respectively), due the high incidence of foetal resorptions in the high dose group (total no. of resorptions of 19, 12, 16 and 69 in controls, 100, 300 and 1000 mg/kg bw/day groups respectively) and with an impact in the total no. of live fetuses (14.0, 13.9, 13.5 and 11.6 in controls, 100, 300 and 1000 mg/kg bw/day groups respectively). These foetal mortalities were not statistically significant but considering the pronounced increase they are considered adverse at 1000 mg/kg bw/day. Other sign of potential developmental toxicity at this dose level was the statistically significant decrease of the bodyweights of live foetuses in both sexes when compared to those in the control group (8% for males and females respectively). However it has to be noted the low magnitude of the variation and the portential correlation with maternal toxicity at this dose level manifested by reduction in bodyweight and bodyweight gain.

External examinations of foetuses did not reveal any malformations that were considered attributable to administration of the test substance. Although shortening of the trunk due to the absence of the lumbar, sacral and caudal vertebrae was found in 2/256 foetuses (2/20 litters) versus 0/323 in control (0/23 litters) in the 1000 mg/kg bw/day group, it is considered unlikely that these changes were treatment-related due to fact that the incidence was low, and moreover this type of malformation is frequently observed in this strain of rats and occurs spontaneously though it has to be noted tha historical control data is not available for this specific lesion. No other external defects were observed at any dose level.

Severe skeletal malformations but not statistically significant were observed in one foetus from dam F82 at 1000 mg/kg bw/day consisting on fusion of all the sternebrae, absence of vertebrae below 2nd lumber vertebra, absence of ribs below 2nd ribs, and absence of coxa. With respect to variations, at this same dose level, splitting of 12th thoracic vertebral body and dumbbell-shaped of 13th thoracic vertebral body were seen in two different foetuses from dams F89 and F92 respectively, but not reaching statistical significance and consequently not considered biologically relevant.

More importantly, the degree of ossification of the metatarsals (variation) was statistically significantly lower in the 1000 mg/kg bw/day dose group (26.6% lower than of controls) and was attributable to test substance administration. On the other hand, 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), and slightly higher but not significant (1.2%) at 300 mg/kg bw/day (2 vs 0 in controls). This observation (11.2% and 1.2% for 1000 and 300 mg/kg bw/day dose group) was higher than historical control data (HCD) for Wistar-Imamichi rats (mean incidences of 0.68%

and 0.61% for 1975-1982[4] and 1983-1986 periods, respectively); although the value of 1.2% exhibited at 300 mg/kg bw/day was within the range of 1983-1986 (0-3.1%) period (unpublished data). 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[5][6][7][8]. Consequently, this effect appears to not be relevant for CLP classification.

Regarding visceral malformations, ventricular septal defect (VSD) showed statistically significant increases at high (litter incidence 35%, foetal incidence 6.6%) and mid (litter incidence 26%, foetal incidence 4%) dose groups, compared with control group (litter incidence 4.3%, foetal incidence 1.3%), respectively. The values of VSD obtained at 1000 and 300 mg/kg/bw/day dose groups are within the normal range when compared to the 1975-1982 historical control data for foetal incidences (range 0-11.3%; mean incidence= 1.47%; 56/3809 foetuses examined)[4], and only the VSD incidence at 1000 mg/kg bw/day is out of the 1983-1986 historical control data for foetal incidences (range 0-5.3%; mean incidence= 1.52%; 42/2757 foetuses examined, unpublished data). Litter incidences for the historical controls were not available.

Additionally, 2/122 foetuses from different 1000 mg/kg bw/day litters (litter incidence 2/20), and one foetus from a litter of 300 mg/kg/day dose group (foetal incidence 1/149, litter incidence 1/23) exhibited renal pelvis and ureter dilation. These foetal incidences were within HCD and thereby considered not relevant for classification. No litter incidences for the historical controls were available.

Futher comparison of this visceral malformations in Imamichi rats with historical control data can be found under section *10.10.6.*

Therefore, a **NOAEL for developmental toxicity** in rats could be established at **300 mg/kg bw/day** based on foetal deaths (reduction in the total number of live pups, increase in the total resorptions and foetal mortality), and delayed in ossified metatarsals observed at 1000 mg/kg bw/day.

NOAEL for maternal toxicity has been established at **100 mg/kg bw/day** based on decreased bodyweight gain and low food consumption rates detected in dams at 300 mg/kg bw/day.

Another developmental toxicity study in rats (Anonymous 27, 1996; B.6.6.2.3) was conducted to assess the potential for maternal and developmental toxicity of flazasulfuron (SL-160 technical) in Sprague-Dawley strain rats. Four study groups of 24 females each were dosed with 0, 100, 300 and 1000 mg/kg bw/day.

No maternal mortality or morbidity signs were observed at any dose level. Regarding clinical observations, an increase in treatment-related incidences such as anogenital stains (25% vs 4% in controls) and alopecia (16% vs 0% in controls), were observed in the high dose group.

Furthermore, in the 1000 mg/kg bw/day group, dams bodyweights were statistically significantly lower from controls from gestation day 9 (6% on day 9 and 12, 7% on day 16 and 6% on day 20), and reduced bodyweight gain (125%) with respect to control was also observed through organogenesis (days 6-9). There was no statistically significant effect on corrected maternal bw at termination or corrected maternal body weight gain over the full study.

Food consumption was statistically significantly lower from days 6-9 (20%) and days 9-12 (13%) in high dose dams though on days 16-20 there was an increase (13%) No significant differences in bodyweight or food consumption were detected in mid and low dose groups.

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Liver weights were recorded from each dam, however, no histopathological examinations were performed in any of the removed livers. Thus, a statistically significant increase of relative liver weights were recorded at high and mid dose groups (10% and 8%, respectively), compared to the control group average, and these changes were attributed to test substance administration. On the other hand, statistically significantly lower absolute gravid uterine weights (15%) were recorded at 1000 mg/kg bw/day dose group. However, MSCA deems that these differences may be due to the decreased bodyweights observed in the high dose group, and a relative gravid uterine weight corrected for bodyweight should have been calculated.

Developmental parameters such as resorptions including rate of litters with resorptions, abortions and number of live and dead foetuses, were not affected at any dose level. However, bodyweights of live foetuses of both sexes were statistically significantly lower in the high dose group (16% lower as compared to controls in both sexes). In the mid dose group, a slight, but not statistically significant decrease in bodyweights (4% lower as compare to controls in both sexes) was detected. However, the mean foetal bodyweights at 300 mg/kg bw/day (3.19 and 3.33 g for females and males respectively) were in males just outside of historical control data range recorded through 1988-1992 period (3.19-3.59 and 3.35-3.77 g for females and males respectively).

External examinations of foetuses did not reveal any malformation attributable to test substance administration, thus the malformations found were considered to be spontaneous events.

Table 81: Summary of foetal external malformations

		0 mg/kg /day	**100 mg/kg /day**	**300 mg/kg /day**	**1000 mg/kg /day**
Litters evaluated	N	23	22	24	23
Fetuses evaluated	N	316	301	341	304
Live	N	316	301	341	303
Dead	N	0	0	0	1
Absence of tail					
Foetal incidence	N(%)	1(0.3)	0	1(0.3)	0
Litter incidence	N(%)	1(4.3)	0	1(4.2)	0
Edematous					
Foetal incidence	N(%)	0	0	0	1(0.3)
Litter incidence	N(%)	0	0	0	1(4.3)
Micrognatia - small lower jaw					
Foetal incidence	N(%)	1(0.3)	0	0	0
Litter incidence	N(%)	1(4.3)	0	0	0
Filamentous tail					
Foetal incidence	N(%)	0	0	0	1(0.3)
Litter incidence	N(%)	0	0	0	1(4.3)
Total external malformations					
Foetal incidence	N(%)	2(0.6)	0	1(0.3)	2(0.7)
Litter incidence	N(%)	2(8.7)	0	1(4.2)	2(8.7)

N= number

Table 82: Summary of foetal external variations

	0 mg/kg /day	**100 mg/kg /day**	**300 mg/kg /day**	**1000 mg/kg /day**

Litters evaluated	N	23	22	24	23
Fetuses evaluated	N	316	301	341	304
Live	N	316	301	341	303
Dead	N	0	0	0	1
Glassy appearance					
Foetal incidence	N(%)	0	0	1(0.3)	0
Litter incidence	N(%)	0	0	1(4.2)	0
Pale in color					
Foetal incidence	N(%)	0	0	0	1(0.3)
Litter incidence	N(%)	0	0	0	1(4.3)
Total external variations					
Foetal incidence	N(%)	0	0	1(0.3)	1(0.3)
Litter incidence	N(%)	0	0	1(4.2)	1(4.3)

N = number

No visceral observations were considered to be treatment-related. Only two instances were detected in treatment groups, which were abnormal course of the aortic arch in one foetus of high dose group, and microphthalmia in another foetus of mid dose group.

Table 83: Summary of foetal visceral malformations

		0 mg/kg /day	**100 mg/kg /day**	**300 mg/kg /day**	**1000 mg/kg /day**
Litters evaluated	**N**	23	22	24	23
Fetuses evaluated	**N**	316	301	341	304
Live	**N**	316	301	341	303
Dead	**N**	0	0	0	1
Situs inversus					
Foetal incidence	**N(%)**	1(0.3)	0	0	0
Litter incidence	**N(%)**	1(4.3)	0	0	0
Microphthalmia					
Foetal incidence	**N(%)**	0	0	1(0.6)	0
Litter incidence	**N(%)**	0	0	1(4.2)	0
Abnormal course of the aortic arch					
Foetal incidence	**N(%)**	0	0	0	1(0.3)
Litter incidence	**N(%)**	0	0	0	1(4.3)
Total visceral malformations					
Foetal incidence	**N(%)**	1(0.3)	0	1(0.3)	1(0.3)
Litter incidence	**N(%)**	1(4.3)	0	1(4.2)	1(4.3)

N= number

Regarding skeletal observations, statistically significantly increased foetal incidences of ossification variations (retarded ossification), or slight ossification irregularities which may or may not be present in the adult specimen, were detected at 1000 mg/kg bw/day group for fetuses (79.5%, 116/146 foetuses examined vs 62.1%, 95/153 foetuses examined in controls) but not for litters (23/23 (100%) at all dose levels vs 21/23 (91.3%) in controls). These ossification variations were most prominent in the vertebrae (incompletely ossified first cervical vertebral transverse processes, split thoracic vertebral centrum, incompletely ossified and unossified sacral vertebral transverse processes, and unossified caudal vertebral transverse processes), and sternebrae (incompletely ossified fourth sternebra, and unossified fifth or sixth sternebra). These findings were attributed to test substance administration and consequently relevant for development.

Table 84: Summary of foetal skeletal malformations

		0 mg/kg /day	**100 mg/kg /day**	**300 mg/kg /day**	**1000 mg/kg /day**
Litters evaluated	**N**	23	22	24	23
Fetuses evaluated	**N**	153	144	166	146
Live	**N**	153	144	166	146
Dead	**N**	0	0	0	0

		0 mg/kg /day	100 mg/kg /day	300 mg/kg /day	1000 mg/kg /day
Exoccipital fused to cervical transverse process					
Foetal incidence	**N(%)**	1(0.7)	0	0	0
Litter incidence	**N(%)**	1(4.3)	0	0	0
Mandible - misshapen					
Foetal incidence	**N(%)**	1(0.7)	0	0	0
Litter incidence	**N(%)**	1(4.3)	0	0	0
Thoracic centra(um) - misshapen					
Foetal incidence	**N(%)**	0	0	0	1(0.7)
Litter incidence	**N(%)**	0	0	0	1(4.3)
Lumbar centrum(A) - misshapen					
Foetal incidence	**N(%)**	0	0	0	1(0.7)
Litter incidence	**N(%)**	0	0	0	1(4.3)
Reduction in number of presacral vertebrae					
Foetal incidence	**N(%)**	1(0.7)	0	1(0.6)	0
Litter incidence	**N(%)**	1(4.3)	0	1(4.2)	0
Sacral centrum(A) - misshapen					
Foetal incidence	**N(%)**	0	0	0	1(0.7)
Litter incidence	**N(%)**	0	0	0	1(4.3)
Absence of sacral and caudal vertebrae					
Foetal incidence	**N(%)**	1(0.7)	0	1(0.6)	1(0.7)
Litter incidence	**N(%)**	1(4.3)	0	1(4.2)	1(4.3)
Rib(s) - fused					
Foetal incidence	**N(%)**	0	0	0	1(0.7)
Litter incidence	**N(%)**	0	0	0	1(4.3)
Total skeletal malformations					
Foetal incidence	**N(%)**	2(1.3)	0	1(0.6)	2(1.4)
Litter incidence	**N(%)**	2(8.7)	0	1(4.2)	2(8.7)

N= number

Therefore, a **NOAEL for developmental toxicity** has been established at **300 mg/kg bw/day** based on decrease foetal bodyweight and retardation in ossification at 1000 mg/kg bw/day dose group.

NOAEL for maternal toxicity has been stabilised at **100 mg/kg bw/day** based on increase maternal relative liver weight at 300 mg/kg bw/day.

The teratogenicity study in rabbits (Anonymous 28, 1988c; B.6.6.2.4) was conducted to assess the potential teratogenicity effect of flazasulfuron (SL-160 technical) in New Zealand White rabbits. Flazasulfuron was tested at dose levels of 0, 50, 150, and 450 mg/kg bw/day, to each of which 17 copulated females were assigned.

The rationale for selection of dose levels arises from a preliminary study in which flazasulfuron was orally administrated to 7 copulated female rabbits per group at dose of 0, 30, 100, 300 and 1000 mg/kg bw/day, from day 6 to day 18 of pregnancy. At the dose of 1000 mg/kg all females stopped eating at 2 days after initiation of administration and did not resume eating until autopsy. Of the seven females in the high dose group, one female died before schedule sacrifice, and two female were killed because they showed clinical signs of sedation, lateral position and signs of abortion. The remaining females survived to the day of cesarean sectioning, however, no live foetuses were obtained. Consequently, a dosage level of 0, 50, 150 and a maximum dose of 450 mg/kg bw/day was established for the definitive study.

In the main study, one female in each of the control, 50 and 450 mg/kg bw/day groups died due to a failure in the substance administration, and between 1-3 animals per group were proved to be non-pregnant (1, 2, 2 and 3 females for 0, 50, 150 and 450 mg/kg bw/day dose groups, respectively). No maternal mortality or morbidity signs related to treatment were detected in any of the study groups. No significant differences in the maternal body weights, body weight gains and food consumption between the control and any of the treated groups were observed.

Table 85: Clinical Findings in Females

Groups	**0 mg/kg bw**	**50 mg/kg bw**	**150 mg/kg bw**	**450 mg/kg bw**
Numbers of animals examined	17	17	17	17
Numbers of pregnant animals	16	15	15	14
Clinical findings				
NAD	12	12	13	7
Found dead	1	1	0	1
Diarrhea	4	3	1	2
Sedation and lateral position	1	0	0	0
Abortion	1	1	1	5

NAD = No abnormalities observed

The incidence of abortions related to treatment was higher in the 450 mg/kg bw/day dose group compared with other tested groups (5/14 pregnant animals at top dose, vs 1/16 pregnant animals in control). All the abortions occurred in the last third of the pregnancy, specifically, through pregnancy days 20-23, and this period is out of the organogenesis period in rabbits (day 6-18). However, in the 450 mg/kg bw/day dose group, 6 females out of 14 (43%) stopped eating after initiation of administration for more than 3 days compared with 3 females of control group (19%). 4 out of the 6 females of the 450 mg/kg bw/day group and 1 out of 3 females of controls were killed due to abortions. The remaining females of both groups were either found dead or killed in extremis. One abortion at 450 mg/kg bw/day was seen without this long period (> 3 days) of eating disruption but it has to be noted that this animal did not eat anything on days 6-7 of treatment. The autopsy of these animals revealed causes of the eating behavior disruption; repletion of the stomach with food and white muddy materials that seemed to be the mixture of food and residue of dosing solution, and emptiness in the intestines. Taking into account the high severity of maternal toxicity with eating disruption in long periods (> 3 days) in dams at 450 mg/kg bw/day, abortions are considered a direct consequence of this maternal toxicity and not regarded for development.

Reproductive data did not show effects attributable to treatment in any parameters examined.

External alternations were no detected in any of foetuses examined at any dose levels, only in controls.

Minor skeletal alterations, non-treatment-related were observed, such as asymmetry of sternebrae in one foetus at 450 mg/kg bw/day, and fusion of the sternebrae in 2 foetuses from 1 litter and 4 foetuses from 2 litters in the 50 and 150 mg/kg groups, respectively.

Visceral examinations only displayed agenesis of the pulmonary trunk in one foetus of 450 mg/kg bw/day (1.8%) dose group and was considered a spontaneous event.

Therefore, a **NOAEL for developmental** has been established > **450 mg/kg bw/day** due to no developmental effects were detected at any dose level.

NOAEL for maternal toxicity has been established at **150 mg/kg bw/day** based on higher incidence of abortions in the high dose group and a higher number of females that stopped eating for more than 3 days after initiation of administration in the 450 mg/kg bw/day dose group.

Summary of other studies relevant for developmental toxicology

Relevant developmental effects in generational studies summarised in section 10.10.1:

Range-finding study in rats (Anonymous 23, 1993; B.6.6.1.1). Test substance was tested at dose levels of 0, 100, 500, 2500 and 10000 ppm (equivalent to 0, 7, 34, 175 and 731 mg/kg bw/day for males and 0, 8, 40, 196 and 791 mg/kg bw/day for females).

Table 86: Mean Weight of Live Offspring (g), F1a Litters

Group, No	Dose, ppm	Day 0 Weight	Day 7 Weight	Day 14 Weight	Day 21 Weight
1	0	6.4 (0.6) n = 16	17.5 (1.7) n = 16	36.0 (2.4) n = 16	58.6 (4.1) n = 16
2	100	6.5 (0.7) n = 12	17.0 (1.9) N = 12	35.5 (3.2) n = 12	59.0 (3.9) n = 12
3	500	6.8 (0.7) n = 14	18.7 (2.1) n = 14	38.3 (2.8) n = 14	63.3* (5.0) n = 14
4	2500	6.6 (0.7) n = 15	17.4 (2.1) n = 15	35.6 (3.0) n = 15	58.5 (4.4) n = 15
5	10000	6.8 (0.7) n = 12	16.7 (1.7) n = 12	33.4* (1.4) n = 12	58.8** (2.9) n = 12

Table 87: Mean litter size at day 0. F1a litters

Dose (ppm)	Liveborn pups	Stillborn pups	Total pups
0	16.5 (1.7) N=16	0.2 (0.4) N=16	16.7 (1.9) N=16
100	15.4 (2.4) N=12	0.3 (0.6) N=12	15.7 (2.6) N=12
500	14.2 (3.2)	0.1 (0.5)	14.4 (3.2)

	N=14	N=14	N=14
2500	**13.8*** (3.4) N=15	0.3 (1.0) N=15	**14.1*** (3.4) N=15
10 000	13.3 (4.2) N=12	0.2 (0.6) N=12	**13.5*** (4.1) N=12

*= Statistically significant difference from control group at 0.05 level (Bartlett´s test)

Table 88: Historical Control Data for number of live births from three rat reproduction studies conducted at Ricerca from 1987 to 1991

Study #	Type	Year	Range For Control # of Live Births
1722-87-0121-TX-003	Two-Generation Reproduction	1987	11.3 to 14.5
1703-88-0176-TX-003	Two-Generation Reproduction	1988	
3822-91-0014-TX-003	One-Generation Reproduction	1991	

Table 89: Historical Control Data for number of live births from three rat reproduction studies conducted atRicerca from 1992 to 1996

Study #	Type	Year	Control # of Live Births	
5330-92-0223-TX-004	Two-Generation Reproduction	1992	F1 = 14.1	**Range** 12.7 - 14.1
			F2 = 12.7	
6755-96-0042-TX-003	Two-Generation Reproduction	1996	F1 = 13.6	
			F2 = 13.0	
6754-96-0041-TX-001	One-Generation Reproduction	1996	13.6	

The pup viability through days 1-21 was not affected by treatment at any dose level. However, the mean weight of the live offspring on day 14 and 21 were statistically significantly lower in the high dose group when compared with the control values (7% and 10% for day 14 and 21, respectively). These significant reductions in pup bodyweight can be due to parental toxicity manifested by significant reductions in bodyweight throughout entire lactation period (7-14%). In the 2500 ppm or less dose groups did not display differences compared with control group.

The significant reduced litter sizes at 2500 and 10000 ppm cannot be univocally addressed to development. Due to lack of data on number of implantations, it is not possible to determine whether this reflects the reduced number of implantations (to be addressed under sexual function and fertility) or postimplantation losses (to be addressed under developmental toxicity) or both. The mean number of live born pups on lactation day 0 was lower in all the treatment groups compared with control group, and showed a dose-related trend. However, the only value that displayed a statistical significant difference was the mean litter size of live born pup of 2500 ppm dose group. Historical control data (HCD) from three previous studies conducted in same institute between 1987 and 1991 displayed a live born pups range of 11.3 to 14.5. Three more studies were further conducted between 1992 and 1996, and the mean litter size of live born pup ranged from 12.7 to 14.1. Thus, it is important to remark that the live born pup mean of control group (16.5) and 100 ppm dose group (15.4) were slightly higher than observed in HCD. Therefore, the differences in the number of live born pups between the

treatment groups and the control group were considered as incidental nature and no treatment-related.

A **NOAEL** for **developmental toxicity** was established in the RAR (2016) at **2500 ppm (175 / 196 mg/kg bw/day for males / females)** based on the decrease in pups weight noted in lactation days 14 and 21 at 10000 ppm dose group.

Two-generation reproductive study in rats (Anonymous 24, 1995b; B.6.6.1.2). Flazasulfuron tested at 0, 200, 2000 and 10000 ppm dose levels (equivalent to 0, 14, 141, and 717 mg/kg bw/day for males and 0, 16, 160, 801 mg/kg bw/day for females, respectively).

Table 90: Mean Maternal Body Weights During Pregnancy

Dietary concentration (ppm)	**Day 0**	**DAY 7**	**Day 14**	**Day 21**
F1a females				
0	310.1	344.5	377.5	445.8
200	295.8	328.8	361.4	435.2
2000	299.2	330.0	361.2	429.4
10000	265.0**	292.5**	321.3**	388.7**
F2a females				
0	300.3	323.6	355.3	424.0
200	284.2	312.3	346.6	414.8
2000	287.6	310.8	343.0	409.3
10000	256.7**	271.3**	296.1**	360.1**

** Statistically significant difference from control group p = < 0.01

Table 91: Mean Maternal Body Weights During Lactation

Dietary concentration **(ppm)**	**Day 0**	**Day 7**	**Day 14**	**Day 21**
F1a females				
0	353.5	361.1	364.8	337.4
200	337.0	345.8*	350.1*	322.2*
2000	339.0	343.1**	346.0**	318.4**
10000	299.5**	307.4**	308.6**	296.9**
F2a females				
0	335.9	343.6	345.2	315.0
200	321.2	331.4	343.4	318.5
2000	323.3	326.2*	332.9	310.6
10000	280.2**	288.8**	298.0**	288.8**

* Statistically significant difference from control group p = < 0.05
** Statistically significant difference from control group p = < 0.01

Table 92: Mean Weights of Live Offspring (g)

Dietary Concentration (ppm)	**Day 0**	**Day 4 Postcull**	**Day 7**	**Day 14**	**Day 21**
F1a litters					

Dietary Concentration (ppm)	**Day 0**	**Day 4 Postcull**	**Day 7**	**Day 14**	**Day 21**
0	6.4	10.7	17.4	35.8	57.5
200	6.3	10.2	17.1	35.0	55.3
2000	6.5	11.1	18.1	35.9	55.8
10000	6.4	9.9	15.6**	30.6**	47.2**
F2a litters					
0	6.5	10.8	17.7	35.5	58.4
200	6.2	10.3	17.0	34.5	55.4
2000	6.3	10.4	17.0	34.3	54.4
10000	6.0*	9.6**	15.2**	30.3**	46.8**

* Statistically significant difference from control group p = < 0.05

** Statistically significant difference from control group p = < 0.01

Additionally it has to be noted that kidney effects observed in parental animals (P and F1) in post-mortem examinations are not considered related with development taking into account the time of the observations and also the duration of dosing during premating and also gestation and lactation covering several weeks. Consequently they are regarded for STOT RE.

Regarding necropsy examinations, relevant kidney findings were mainly detected in P and F_1 male animals of the high dose group, these being more evident at F_1 generation. An increase in kidney bilateral discoloration incidences were detected in 27% and 53% of P and F_1 male animals, respectively. Moreover, in the F_1 parental animals, it is important to highlight the increase of enlarged observations of both kidneys (87%) together with a high incidence of dilated pelvis and grainy appearance (50% and 43%, respectively). Minor incidences of both pale kidneys were also observed (27% and 10% for P and F_1 parental male animals, respectively).

Further histopathological analysis confirmed necropsy findings. In the P male adults, an increase in incidence and severity of nephropathy was noted in high dose group (56% and 23% of slight/mild and moderate nephropathy incidences, respectively) compared with other dose groups. Additionally, in same P generation, an important increase of minimal tubular dilation incidences were recorded at high and mid dose groups (63% and 70%, respectively), whereas slight/mild tubular alterations were mainly detected in 10000 ppm males (30%). The dilation of the convoluted tubules was associated with an increase in the diameter of the tubules, but no change in cellular morphology. The study described that the kidneys of males in the high dose group were noticeably larger than those of other groups, and theorised that the increase in luminar diameter may have contributed to the increased size. No kidney alterations were described in P females in any dose group.

Regarding F_1 parental animals, treated adult males showed more high grades of nephropathies (moderate and severe), than females (mostly slight/mild grade). Moderate nephropathies incidences were detected in 30% and 60% of males at high and mid dose groups, respectively, whereas a moderately severe grade was recorded in 67% of 10000 ppm parental males. In contrast, only a relevant increase of 33% of minimal and slight/mild nephropathies incidences were observed in females at high dose group compared with control group.

Similar distribution of other kidney findings as tubular dilation and cystic tubules were observed in F_1 parental animals. At high dose group, most males exhibited severe grades of tubular dilation and cystic tubules, whereas in the mid dose group, F_1 males exhibited moderate grades of tubular dilation and cystic tubules incidences. Besides, congestion of the veins in the renal medulla was also noted in 47% of high dose males, but not in males or females from any other group. This lesion was characterised by dilation of the veins draining the renal pelvis and may have resulted from pressure caused by the tubular dilation.

In addition, F_1 females at the high dose group mostly developed moderate/severe grades of tubular dilation. Conversely, cystic tubules incidences displayed low severity grades. At 2000 ppm or low dose groups, no relevant incidences of both tubular dilation and cystic tubules were found in F_1 females.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FLAZASULFURON (ISO): 1-(4,6-DIMETHOXYPYRIMIDIN -2-YL)-3-(3-TRIFLUOROMETHYL-2-PYRIDYLSULFONYL)UREA

Table 93: Incidence of gross kidney findings in F_0 males

Incidence in group				
Dose level	0 ppm	200 ppm	2000 ppm	10000 ppm
Nº of animals examined	30	30	30	30
Kidney findings				
Bilateral discoloration	0	1	3	8
Both enlarged	1	0	0	3
Right granular	0	0	1	0
Both mottled	1	1	2	2
Both pale	0	1	3	8
Dilated pelvis(es)	0	0	3	4

Table 94: Incidence of gross kidney findings in F_1 males

Dose level	**0 ppm**	**200 ppm**	**2000 ppm**	**10000 ppm**
Kidney finding				
No. of animal examined	**30**	**30**	**30**	**30**
Bilateral discoloration	0	0	4	16
Both enlarged	0	0	5	26
Right granular	0	0	0	13
Both mottled	1	0	1	2
Both pale	0	0	1	3
Dilated pelvis(es)	5	4	7	15

Table 95: Summary of microscopic kidney lesions in F_0 males

Incidence in group				
Dose level	0 ppm	200 ppm	2000 ppm	10000 ppm
Nº of animals examined	30	30	30	30
Kidney findings				
Nephropathy	18	25	28	30
Minimal	14	17	22	5
Slight/mild	4	7	2	17
Moderate	0	1	3	7
Moderately severe	0	0	1	1
Tubular dilation	0	0	22	28
Minimal	0	0	21	19
Slight/mild	0	0	1	9

Table 96:Summary of Nephropathy in F_1 Males and Females

Dose level	**0 ppm**	**200 ppm**	**2000 ppm**	**10000 ppm**
Male kidney findings				
No. of animals Examined	30	30	30	30

Dose level	**0 ppm**	**200 ppm**	**2000 ppm**	**10000 ppm**
Nephropathy	17	23	29	30
- Minimal	11	20	3	0
- Slight/mild	5	3	6	0
- Moderate	1	0	18	9
Moderately Severe	0	0	2	20
Severe/high	0	0	0	1
Female kidney findings				
No. of animals Examined	30	30	30	30
Nephropathy	4	6	9	26
- Minimal	4	6	8	14
- Slight/mild	0	0	1	9
- Moderate	0	0	0	1
Moderately Severe	0	0	0	2
Severe/high	0	0	0	0

Table 97: Summary of microscopic kidney lesions in F_1 males and females

Incidence in group				
Males	**0 ppm**	**200 ppm**	**2000 ppm**	**10000 ppm**
No. of animal examined	30	30	30	30
Tubular dilatation	0	1	0	0
Minimal	0	1	0	0
Slight/mild	0	0	2	0
Moderate	0	0	15	4
Moderately severe	0	0	11	14
Severe/high	0	0	2	12
Cystic tubules	0	0	11	30
Minimal	0	0	1	0
Slight/mild	0	0	6	2
Moderate	0	0	4	13
Moderately severe	0	0	0	5
Severe/hight	0	0	0	10
Females				
No. of animals examined	**30**	**30**	**30**	**30**
Tubular dilatation	0	1	4	30
Minimal	0	1	3	0
Slight/mild	0	0	1	3

Incidence in group				
Moderate	0	0	0	6
Moderately severe	0	0	0	15
Severe/high	0	0	0	6
Cystic tubules	0	0	0	21
Minimal	0	0	0	6
Slight/mild	0	0	0	10
Moderate	0	0	0	5
Moderately severe	0	0	0	0
Severe/high	0	0	0	0

Furthermore, no treatment-related differences in the number of live born or stillborn pups per litter were noted in the treated groups compared with control group.

Bodyweight of F_1 offspring was decreased in a statistically significant manner from lactation day 7 (10-18%) in the high dose group. F_2 offspring of this high dose group exhibited lower bodyweights throughout entire lactation period (8-20%). By contrast, bodyweights of both F_1 and F_2 litters at 2000 ppm dose level and less did not show differences compared with control group. These decreases could be associated with maternal toxicity considering the significant reductions in bodyweight in both P and F_1 parental dams during gestation and lactation. By contrast, bodyweights of both F_1 and F_2 litters at 2000 ppm dose level and less did not show differences compared with control group.

Regarding F_1 and F_2 offspring necropsy examination, no malformations in any organ including kidney were associated to test substance administration.

Therefore, a **NOAEL** for **developmental toxicity** was established in RAR (2016) at **2000 ppm (141 / 160 mg/kg bw/day for males / females)** based on the decreased pups weights noted in F_1 and F_2 litters during lactation at 10000 ppm dose group.

Relevance of ventricular septal defect for classification and labelling

Ventricular septal defect (VSD) incidences observed in Wistar-Imamichi rats teratogenicity study (Anonymous 26, 1988b) presents uncertainties regarding a potential classification as toxic to development. Thereafter, and listed in chronological order, MSCA summarises the studies presented by applicant to assess the relevance of VSD for classification and labelling.

Anonymous 29, 1983

Foetal incidences of historical control data comprised 1975-1982 period from Wistar-Imamichi (WI) and Sprague-Dawley (SD) rat strains are presented in this study. Spontaneous VSD foetal incidences in Wistar-Imamichi rat strains presented a mean value of 1.47% (56/3809 foetuses examined) with a wide occurrence range (0-11.3%). Otherwise, low spontaneously developed VSD incidences were observed in Sprague-Dawley rats (mean incidence= 0.3%; 3/991 foetuses examined; range 0-1.8%). These data were further complemented with the data obtained from same laboratory during the period 1983-1986 (unpublished data) in Wistar-Imamichi rat strain (mean foetal incidence=1.52%; 42/2757 foetuses examined; range 0-5.3%). No historical control data for litter incidences was available.

On the other hand, the historical control data showed high foetal incidences of other visceral malformations were observed for both rats strains such as renal pelvis dilation (mean incidence= 2%; 79/3809 foetuses examined; range 0-5.6% in WI and mean incidence= 8.2%; 82/991 foetuses examined; range 2.5-13.5% in SD, respectively) and hydroureter (mean incidence= 6.1%; 233/3809 foetuses examined; range 0-25.2% in WI and mean incidence= 19.7%; 196/991 foetuses examined; range 0-54.5% in SD, respectively). It is important to note that renal pelvis dilation and hydroureter alterations in offspring were subsequently described in a teratogenicity study in Wistar-Imamichi pregnant rats treated with flazasulfuron (Anonymous 26, 1988b). In

particular, a mean foetal incidence of 1.6% and 0.7% were recorded in the 1000 and 300 mg/kg bw/day dose groups, respectively. No historical control data for the litter incidences were available.

Anonymous 30, 1984

This short report described temporal changes of VSD foetal incidences in Wistar-Imamichi foetal and breast-fed rats. The study analysed through daily observation the foetal incidences of VSD from 20 days of gestation to 14 days of postnatal age. The most important finding showed a decrease of VSD foetal incidences from 7.6% at day 20 of gestation to 3.01% and 0% at day 21 of gestation and at day 1 of postnatal age, respectively. These observations showed that VSD disappeared during the postnatal development process, and were considered temporal features previous to septal closure. Besides, authors accomplished a retrospective study using control animals in teratogenicity studies from 1981 to 1984 (8 studies, 20 rats per study). The derived data of these VSD incidences at 21 days of gestation (mean incidence=2.9%; range 0-11%) confirmed the mean incidence and range previously described on different control datasets.

Anonymous 31, 1987

Teratogenicity effects of ephedrine on the foetal heart were tested in Wistar-Imamichi rats at 0, 0.1, 1, 10 and 50 mg/kg bw/day dose levels. Pregnant females received a single intraperitoneal injection of ephedrine on day 9, 10 or 11 of pregnancy. Statistically significant differences in mean VSD foetal incidences at 20 days of gestation were detected in females at 1, 10 and 50 mg/kg bw/day dose groups (mean foetal incidence= 15.7%, 21.7% and 27.2% for 1, 10 and 50 mg/kg bw/day dose groups, respectively), compared with control group (3.2%). The authors concluded that ephedrine administration to rats during early stages of pregnancy affects their foetuses and causes cardiovascular malformations.

Anonymous 32, 1997

This research work analysed the incidences of spontaneous heart alterations in controls Sprague-Dawley (SD) rats at several prenatal and postnatal stages. The authors described specific alterations of the cardiac membranous ventricular septum using macrodissection, scanning electron and light microscopy techniques in foetal (day 17, 19 and 21), weanling (21 day postnatal), and adult (5-10 months old) SD rats. The ventricular septum is formed by muscle tissue originating from the floor of the ventricle (muscular portion of septum) and from atrioventricular endocardial cushions (membranous portion of septum). The membranous part of the ventricular septum is the most common location of ventricular defects. The most common observation in all groups was a non-patent depression (NPD) in the membranous septum. Non-patent depressions were characterised by a split in the endocardial cushion cells in the atrioventricular component of the septum, without obvious streaming of blood from right ventricle. They persisted postnatally as a blind-ended diverticulum directed above the tricuspid valve, and were typically triangular, oval, or slit-like in appearance with a diameter of 40 to 200 μm.

NPD were observed at a higher mean incidence than VSDs in foetuses at 21 days (4.3% vs. 2.0%, respectively), and, importantly, membranous VSD were neither observed in weanling or adult hearts, nor in foetus at days 17 or 19. In addition, not a single case of a muscular VSD was observed in any of examined animals.

In order to determine if there were morphological differences between ventricular septal alterations occurring spontaneously and those induced by cardiovascular teratogens, trimethadione and trypan blue were used as positive controls. Trimethadione produced a 93% of VSD mean incidences and none of NPD at 21 days of gestation. The treatment-induced VSDs were much larger than those occurring spontaneously, but were similar histologically.

In contrast, trypan blue generated low mean incidences of VSD and NPD at same pregnant stage (9.2% and 9.9%, respectively). Again, neither trimethadione nor trypan blue produced muscular VSDs although in some cases patencies appeared to be perimembranous. Both substances produced small foetuses on day 21 of gestation, and it was suggested that alterations of the ventricular septum were associated with intrauterine growth retardation (IUGR). To determine if IUGR is necessarily associated with alterations of the ventricular septum in SD rats, authors analysed the effect of diet restriction in heart and skeletal development at day 21 of gestation. Similar low mean incidences of NPD and VSD were detected in both *ad libitum* and diet-restricted groups. NPD observations were detected in 1.7% and 3.5% of foetuses in *ad libitum* and diet-restricted groups, respectively; whereas VSD observations were recorded in 1.6% and 1.3% of foetuses in *ad libitum* and diet-

restricted group, respectively. These findings indicated that IUGR is not necessarily associated with alterations in the development of the ventricular septum in this rat strain. Moreover, several skeletal variations as sign of IUGR were detected in diet-restricted group.

Therefore, spontaneous VSD incidences were detected at prenatal stage, but were subsequently decreasing at postnatal stage and disappeared in weanling or adult animals. Same observation was detected in *ad libitum* and diet-restricted rats. Besides, trimethadione treatment showed that VSD incidences were so much higher and 5-time longer that occurred spontaneously in controls. This result determines that a cardiovascular teratogenic substance increases considerably the VSD incidences compared with spontaneously VSD incidences detected in control foetuses. Taken together, these data suggest that VSD is not a symptom of IUGR, and the low incidences observed in non-treated rats could be considered as normal events of the development processes occurred in prenatal stages that close spontaneously during neonatal life.

Anonymous 33, 2002

This study used transthoracic echocardiography and Doppler techniques to define congenital heart defects in commercial Wistar-Kyoto (WKY) strain rats. The authors used this rat strain because represents the most appropriate normotensive counterpart to the spontaneously hypertensive rat (SHR). WKY rats were purchased from two different companies and the presence of cardiac defects was checked. The 22% (15/68 animals) of examined rats exhibited VSD and 6% (4/68 animals) of examined rats presented other heart alterations different to VSD such as pulmonary stenosis (mean incidence= 3%; 2/68 examined rats), patent ductus arteriosus (mean incidence=1.5%, 1/68 examined rats) and aortic regurgitation (mean incidence= 1.5%, 1/68 examined rats). The pathologic examination of heart revealed that right ventricle was hypertrophied and dilated. Besides, right ventricular weight was greater in rats with septal defect (180%) than in normal rats, and the ventricle output was significantly greater than the left in the VSD rats (due to both VSD and pulmonary regurgitation).

Turner, S. W. *et al*, 2002

This is a prospective cohort study were 290 children born between 1991 and 1993 with isolated ventricular septal defect (VSD) were followed up during a mean of 65 months. Of the 290 cases, 227 (78%), 43 (15%) and 20 (7%) of cases were classified as small, moderate and large size VSD, respectively. On the other hand, muscular VSD was presented in 180 cases (62%), whereas 107 (37%) cases were perimembranous, and in 3 cases (1%), the exact morphology was not stated. Spontaneous closure occurred in 155 of total cases (40%), most of them in the children with small VSD (144 cases), 11 cases in moderate VSD size group, and none in kids with large VSD.

Furthermore, muscular VSD was the predominantly form of small VSD (167 cases vs 59 perimembranous VSD), whereas perimembranous VSD was the most common event in moderate (33 cases vs 10 muscular VSD) and large cases (15 cases vs 3 muscular VSD).

The morphology of the lesion had a relevant role in the spontaneous closure of VSD. Of the 155 cases that closed spontaneously, 123 (80%) cases were muscular and 115 of them belonging to small size category. In contrast, only 31 cases (20%) that closed spontaneously pertain to perimembranous VSD group. Kaplan-Meier log-rank analysis confirmed that the incidence of spontaneous closure was significantly greater for muscular compared with perimembranous defects after excluding cases closed surgically ($p < 0.002$). Besides, Kaplan-Meier survival curve showed that a defect of any size in the muscular septum in a child presenting in the first three months of life was over 20 times more likely to close spontaneously than a perimembranous defect of any size that presented at a later age. Although the spontaneous rate of closure for muscular defects reduces with increasing age, there were some defects that closed after seven years of age. Thus, this cohort study provided support for a conservative clinical strategy for muscular defects, as there is a high incidence of spontaneous closure without complications. The size of the defect was the best prognostic indicator of both future surgical and spontaneous closure, whereas the morphology was a strong predictor for these outcomes.

Anonymous 34, 2004

This study analysed the presence of VSD incidences throughout postnatal development after treatment with the cardiovascular teratogen Trimethadione (TMD). TMD administration on gestation days (GD) 9 and 10 has

been shown to induce a membranous VSD. The VSD alterations induced by TMD were larger in size, but morphologically similar to spontaneously arising VSD[9].

Thus, TMD was tested at dose levels of 0, 400 and 600 mg/kg bw/day, in which 40 mated females were assigned to each dose group, whereas 48 mated females were included in the control group.

Neither mortality nor morbidity signs, clinical signs or bodyweight changes in dams were observed in any dose group.

Regarding reproductive toxicity, no statistically significant, but treatment-related decrease on foetal bodyweight was detected at gestation day 21 (7% and 5% in high and mid dose groups, respectively) compared with controls. Besides, mean percent of postimplantation loss was increased in both treated groups when compared with controls (mean incidence= 7.2% and 4.3% in high and mid dose groups, respectively vs 2.9% in controls), although only was statistically significant in the high dose group. The increase in postimplantation loss in the 400 mg/kg bw/day dose group was primarily due to one dam that had three dead foetuses increasing the mean percent of dead foetuses to 1.2 compared to controls, which had none. In rats, the presence of multiple dead foetuses in one litter is an unusual event and this incidence was likely related to treatment. On the other hand, in the high dose group, no statistically significant, but treatment-related increase in early resorptions (mean incidence= 4.7% vs 2.4% in controls), late resorptions (mean incidence= 2.1% vs 0.6% in controls) and death foetuses (mean incidence= 0.4% vs 0% in controls) were noted.

Visceral examination revealed statistically significant increase of isolated VSD incidences per litter at gestation day 21 in the TMD-treated groups (mean incidence= 39.6% and 7.6% in 600 and 400 mg/kg bw/day dose groups, respectively) compared with control group (mean incidence= 0.6%). The VSD incidences in controls and mid dose group were the only cardiac anomaly recorded in the affected foetuses. These VSD were small in size and initially detected by the presence of blood flowing through the defect from the right ventricle. However, at 600 mg/kg bw/day dose group, the VSD still located in the membranous portion of the septum, but were larger and more readily detected without the need to examine the blood flow. In addition, in the 600 mg/kg bw/day dose group, the 10.1% of foetuses presented other vessel anomalies (predominantly persistent truncus arteriosus) associated with VSD.

Afterwards, heart examinations at postnatal (PND) day 21 showed a much lower VSD incidences per litter (mean incidence= 0.03%, 0 and 6.4% for control, mid and high dose groups, respectively) compared with the previously detected ones on the GD 21. It is important to notice that statistically significant decrease in the percent of pup survival occurred in the high dose group during the periods of PND 0–1 and PND 1–4 (mean incidence= 20% and 14%, respectively) compared with the control group. Thus, the percentage of dead pups between the birth and the PND 21 were 3%, 6% and 27% for the control, mid and high dose groups, respectively. This increase in pup mortality in the high dose group clearly accounted for a portion of the decrease in pups with isolated VSD on postnatal day 21

Therefore, the VSD detected on GD 21 in the mid dose group were not present on PND 21, similar to previous study for closure of spontaneously formed membranous VSD in rats (Anonymous 32, 1997). On the other hand, at 600 mg/kg bw/day dose group, a mean VSD incidences of 6.4% per litter were still present on PND 21. These incidences were qualitatively smaller than those noted in the foetuses examined on GD 21, and appeared to be in the process of closure. Some treatment-induced membranous VSD will close during postnatal development similar to spontaneously occurring membranous VSD. Besides, the presence of isolated VSD was not associated with a decrease in foetal weight, suggesting that VSD is a developmental delay not associated with a decrease in bodyweight. Thus, small and isolated VSD do not seem to impact postnatal viability and growth; however, large VSD are likely to affect postnatal survival.

Anonymous 35, 2019

This non-published study reviewed the VSD incidences described on different rat strains in both control animals or after flazasulfuron and cardiovascular teratogens treatment.

[9] Veuthey S, Pexieder T and Scott WJ. (1990). Pathogenesis of cardiac anomalies induced by trimethadione in the rat. In: Developmental cardiology:morphogenesis and function. Futura Publishing Co.Inc. p 453–465.

Firstly, authors review the three main studies in which potential teratogenic effect of flazasulfuron was tested in two rat strains and rabbits. Increase VSD incidences detected in Wistar-Imamichi rats after treatment with flazasulfuron (Anonymous 26, 1988b) produced a concern about a potential teratogenic effect on cardiac development during foetal stages. There was a statistically significant increase in VSD incidences at the 300 and 1000 mg/kg bw/day dose levels (foetal incidence 4% and 6.6%, respectively, litter incidence 35 and 26%, respectively). In the control group, residual, but noticeable VSD mean incidences were also detected (mean foetal incidence 1.3%, litter incidence 4.3%). At 1000 mg/kg bw/day dose group, VSD foetal incidences were slightly higher than historical control data (HCD) from 1983-1986 (range 0-5.3%) and within the range of historical control from 1975-1982 period (range 0-11.3%). The earlier HCD, however, was outside the accepted 5 year window. At 300 mg/kg bw/day dose group, a higher incidence of VSD was also observed, but the foetal incidence fell into the range of the HCD obtained in this laboratory. Similar results were obtained in the preliminary study conducted by same laboratory (mean incidence= 1.7%, 0% and 6.8% of VSD foetal incidences in 0, 50 and 1000 mg/kg bw/day dose groups, respectively). Historical control data for the litter incidences was not available.

In the following study in Sprague-Dawley rats (Anonymous 27, 1996), flazasulfuron induced maternal toxicity at 1000 mg/kg bw/day, specifically, statistically significant decrease in bodyweights compared with controls. In addition, in both 300 and 1000 mg/kg bw/day dose groups, an increase in maternal liver weight relative to bodyweight was recorded, and this observation was attributable to test substance administration. Furthermore, decreased foetal bodyweight, and retardation in ossification were noted in the high dose group. However, there was no evidence of VSD incidences at any dose level.

The potential teratogenic effect of flazasulfuron was further evaluated in Zealand White rabbits (Anonymous 28, 1988c). As a result of maternal toxicity, higher incidence of abortions and a higher number of females that stopped eating for more than 3 days after initiation of administration were detected in the high dose group of 450 mg/kg bw/day. Likewise, no VSD incidences were detected at any dose level.

Detailed analysis of VSD incidences in control rat strains and their evolution during postnatal development were further conducted. In 1984, a study analysed the VSD foetal incidences described on previous studies (Anonymous 30, 1984). Examination of 2444 foetuses revealed a high incidence of VSD, renal pelvis dilation and hydroureter in Wistar-Imamichi rat strain (3.01%, 1.35% and 5.48%, respectively). The authors also investigated the occurrence of ventricular septal defects in foetal and breast-fed rats from 20 days of gestation to 14 days of postnatal age. This analysis showed that VSD incidences were reduced from 7.6% from day 20 gestation, to 3.01% at day 21 of gestation and no VSD were recorded at 1 day of postnatal age or older. The authors conclude that results demonstrate that VSD develops in up to approximately 10% of Wistar-Imamichi foetal rats between late pregnancy through the early postnatal period.

On the other hand, the authors of this review compiled the HCD from Sprague-Dawley rats that Charles River regularly publishes for Development and Reproductive Toxicity Studies. According data from Charles River laboratories, for the Wistar-Imamichi rat strain, the average foetal incidence of VSD was 0.08% (range 0-1.0%) and for Sprague-Dawley (Crl:CD BR) rats the average foetal incidence was 0.03% (range 0-0.6%). The authors showed that VSD incidences detected in Wistar-Imamichi rats from Imamichi Institute (mean foetal VSD incidence of 1.47% and 1.52% for 1975-1982 and 1983-1986 periods, respectively) were 20-100 folds higher that VSD incidences detected in same rat strain and in Sprague-Dawley rats from Charles River Company. The review remarks that the interpretation of VSD data from Wistar-Imamichi strain is very difficult to interpret and far greater weight should be placed on the study in Sprague-Dawley strain rats.

Furthermore, a VSD evolution from gestation day (GD) 17 to postnatal day (PND) 21 on Sprague-Dawley rats was analysed (Anonymous 32, 1997). Membranous VSD incidences were detected in 2.0% of the foetuses examined on GD 21, but in none of the offspring at postnatal day 21 or in adult rats. Non-patent depressions of the membranous ventricular septum (i.e. no hole present), were observed at slightly higher incidence (4.3%) on GD 21 and in a similar incidence on PND 21 and in adult animals (11.8% and 9.1% in PND 21 and adult rats, respectively). Pre-term foetuses examined on GD 17 had 8.9-fold higher incidences of non-patent depressions. Besides, no muscular VSD was observed.

Afterwards, similar VSD evolution was detected after Trimethadione treatment on Sprague-Dawley rats. VSD incidences on foetuses on GD 21 were 0.6%, 7.6% and 39.6% in the control, low and high dose, respectively.

Foetuses examination on PND 21 displayed VSD incidences of 0.03%, 0.0% and 6.4% in the control, low and high dose, respectively. Although decreased postnatal survival contributed to the decreased incidences of VSD on PND 21, it was shown, that the less pronounced VSD closed and that the diameter of VSD observed on PND 21 were smaller compared to GD 21. These findings indicate that VSD were in the process of closure, which is in concordance to the situation described for humans, where the size of VSD decreases with age (Turner S. W. *et al*, 2002).

The review strongly supports the view that the incidence of VSD in Wistar-Imamichi rat is very high and variable. This further difficult the proper interpretation of VSD effects derived from a test substance administration, due to the high background rate such a malformation would not be detected in this strain of rat. Taken together, it is considered that only the results of the developmental toxicity study with flazasulfuron in Sprague-Dawley (CD-Crl :(SD) BR) rat strain should be considered for the purposes of classification. In this study foetal effects were confined to reduced foetal weights and retardation in ossification seen in the high dose group.

10.10.6 Comparison with the CLP criteria

Flazasulfuron presents uncertainties regarding a potential classification as toxic to reproduction category 2 (H361d) due to an increase in ventricular septal defect (VSD) incidences observed in the teratogenicity studies conducted on Wistar-Imamichi rats (Anonymous 25, 1988a and Anonymous 26, 1988b).

CLP criteria regarding reproductive toxicity (which includes adverse effects on development) has already been described in *section 10.10.3* of this document.

No human information is available on the effects of flazasulfuron on development, but there is information from three reliable developmental studies conducted in rat and rabbit.

Firstly, developmental effects different than VSD are discussed and afterwards the assessment on VSD.

Developmental effects apart from VSD

Single generation study in Sprague Dawley rats (range finding study; Anonymous 23, 1993):

Maternal toxicity: At 10000 ppm (731 and 791 mg/kg bw/day for males and females, respectively) significant reduction in bodyweight (16-17% in males and 10-12% in females) and bodyweight gain (25% in males and 27% in females) throughout the premating period, decrese in bodyweight in female dams throughout whole gestation period (13-15%) and the entire lactation period (7-14%) and food consumption reduction during premating period (14-16% for males and 9-15% for females, respectively).

At 2500 ppm (175 and 196 mg/kg bw/day for males and females, respectively), the male bodyweight (7-10%) and bodyweight gains (14%) were lower controls during premating. For females, the difference in bodyweight did not reach statistical significance for any week, but the mean bodyweight gain was statistically lower (15%) throughout this premating period. Reduction in bodyweight was also noted from gestation day 7 (7-8%).

Developmental effects

Not relevant for classification:

At 10000 ppm (731 and 791 mg/kg bw/day for males and females, respectively) decreased pup weight on lactation days 14 and 21 (7% and 10% respectively) at 10000 ppm. The effect has a low magnitude of variation and it could be associated to maternal toxicity manifested by significant reduction in bodyweight of dams throughout entire lactation period (7-14%). It has to be noted that there were no other remarkable developmental effects in this range-finding study. Consequently, it is not regarded for classification.

Statistically significantly reduced litter sizes at 2500 and 10000 ppm. The mean litter size of live born pups was 16.5, 15.4, 14.2, 13.8 and 13.3 in the control, 100, 500, 2500 and 10000 ppm dose groups, respectively, and due to lack of data on number of implantations, it is not possible to determine whether this reflects the reduced number of implantations (to be addressed under sexual function and fertility) or postimplantation losses (to be addressed under developmental toxicity) or both. As noted, the mean number of live born pups on lactation day 0 was lower in all the treatment groups compared with control

group, and showed a dose-related trend. However, the only value that displayed a statistical significant difference was the mean litter size of live born pup of 2500 ppm dose group. Historical control data (HCD) from three previous studies conducted in same institute between 1987 and 1991 displayed a live born pups range of 11.3 to 14.5. Three more studies were further conducted between 1992 and 1996, and the mean litter size of live born pup ranged from 12.7 to 14.1. Thus, it is important to remark that the live born pup mean of control group (16.5) and 100 ppm dose group (15.4) were slightly higher than observed in HCD. Therefore, the differences in the number of live born pups between the treatment groups and the control group were considered as incidental nature and no treatment-related.

2-generation study in Sprague Dawley rats (Anonymous 24, 1995b)

Maternal toxicity: at 10000 ppm (717 and 801 mg/kg bw/day for males and females, respectively), throughout premating period (growth phase), statistically significant differences (>10%) in bodyweights and bodyweight gain for both sexes in parental P and F_1 animals. In addition, reduced bodyweights (>10%) were subsequently described throughout whole gestation and lactation periods in both P and F_1 parental dams at high dose groups. Absolute food consumption was statistically significantly lower in 10000 ppm P males (14-43%) and females (7-35%) during premating period. Similar results were observed in F_1 males (19-21%) and females (12-17%). There was also reductions in bodyweight and bodyweight gain at 2500 ppm (141 and 160 mg/kg bw/day for males and females, respectively). It has to be noted that the NOAEL for maternal toxicity was 250 ppm (14 and 16 mg/kg bw/day for males and females, respectively) based on kidney alterations observed at upper dose levels.

Developmental effects

Not relevant for classification:

At 10000 ppm (717 and 801 mg/kg bw/day for males and females, respectively) bodyweight of F_1 offspring was decreased in a statistically significant manner from lactation day 7 (10-18%) in the high dose group. F_2 offspring of this high dose group exhibited lower bodyweights throughout entire lactation period (8-20%). These decreases could be associated with maternal toxicity considering the significant reductions in bodyweight in both P and F_1 parental dams during gestation and lactation. Consequently, it is not regarded for classification.

Preliminary study in Wistar Imamichi rats (Anonymous 25, 1988a)

Maternal toxicity: At 1000 mg/kg bw/day significant reduction in bodyweights (gestation days 9 (5%), 12 (6%) and 18 (6%)), reduced bodyweight gain (145%, days 6-9) and food consumption decrease on days 6-9 (37%) and 9-12 (30%). At 500 mg/kg bw/day dose group significant decrease of bodyweight gain on days 6-9 (64%).

Developmental effec

Not relevant for classification:

Reduction in the mean foetal bodyweight significantly (4% and 6% for females and males, respectively) at the highest dose level of 1000 mg/kg bw/day in a teratogenicity study in Wistar Imamichi rats. This reduction is of doubtful toxicological relevance taking into account the absence of a clear dose-dependancy and the low magnitude of the reduction. It is not regarded for classification.

Relevant for classification:

Foetal mortality was 19.4% at the top dose as compared to 4.8% in control, and mean number of live foetuses per dam was 11.6 at the top dose as compared to 14.9 in control. These effects on pup survival were observed in presence of maternal toxicity manifested by reduction in bodyweight, bodyweight gain and food consumption. It is remarkable the decrease (145%) in bodyweight gain on days 6-9 of treatment corresponding to organogenesis. However, taking into account the high increase in resoprtions, it is not clear that this effect can be associated to maternal toxicity and it is considered relevant for classification.

Teratogenicity study in Wistar Imamichi rats (Anonymous 26, 1988b)

Maternal toxicity: At 1000 mg/kg bw/day significant reduction of bodyweight from gestation day 9, reduced bodyweight gain on days 6-9 (150%) and days 12-15 (21%). At 300 mg/kg bw/day reduced bodyweight gain on days 6-9 (50%) .

Developmental effects

Not relevant for classification:

1000 mg/kg bw/ day: Statistically significant decrease of the bodyweight of live foetuses of both sexes when compared to those in the control group (8% for both sexesc with maternal toxicity at this dose level manifested by reduction in bodyweight and bodyweight gain.

1000 mg/kg bw/day: 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.

1000 and 300 mg/kg bw/day: 2/122 foetuses from different 1000 mg/kg bw/day litters (litter incidence 2/20), and one foetus from a litter of 300 mg/kg/day dose group (foetal incidence 1/149, litter incidence 1/23) exhibited renal pelvis and ureter dilation. These foetal incidences were within HCD and thereby considered not relevant for classification. No litter incidences for the historical controls were available.

Relevant for classification:

1000 mg/kg bw/ day: Slight increase in the foetal mortality (20.8% vs 5.5%, 3.7% and 4.7% in controls, 100, 300 mg/kg bw/day groups respectively) due the high incidence of foetal resorptions in the high dose group (total no. of resorptions of 19, 12, 16 and 69 in controls, 100, 300 and 1000 mg/kg bw/day groups respectively) and with an impact in the total no. of live fetuses (14.0, 13.9, 13.5 and 11.6 in controls, 100, 300 and 1000 mg/kg bw/day groups respectively). These reductions were not statistically significant but considering the pronounced decrease are regarded adverse, treatment related and relevant for classification.

1000 mg/kg bw/day: The degree of ossification of the metatarsals (variation) was statistically significantly lower (26.6% lower than of controls) and was attributable to test substance administration and not a consequence of maternal toxicity. Consequently, this effect appears to not be relevant for CLP classification.

Teratogenicity study in Sprague Dawley rats (Anonymous 27, 1996)

Maternal toxicity: at 1000 mg kg/bw/day increases in anogenital stains (25% vs 4% in controls) and alopecia (16% vs 0% in controls), reduction in bodyweight from gestation day 9 (6% on day 9 and 12, 7% on day 16 and 6% on day 20), bodyweight gain reduction on days 6-9 (125%) and decreases in food consumption from days 6-9 (20%) and days 9-12 (13%) in high dose dams though on days 16-20 there was an increase (13%). Additionaly it was observed at this dose level significantly lower absolute gravid uterine weights (15%) though MSCA deems that these differences may be due to the decreased bodyweights observed in the high dose group, and a relative gravid uterine weight corrected for bodyweight should have been calculated.

There was also increase of relative liver weights at 1000 and 300 mg/kg bw/day (10% and 8%, respectively) attributed to test substance administration. Histopathological examination not available for liver.

Developmental effects

Not relevant for classification:

1000 mg/kg bw/day: Significant reduction in the bodyweights of live foetuses of both sexes (16% for both sexes). This reduction in fetal weight can be attributed to test administration but could be a direct consequence of the clear maternal toxicity at this dose level.

Relevant for classification:

1000 mg/kg bw/day: statistically significantly increased foetal incidences of ossification variations (retarded ossification), or slight ossification irregularities which may or may not be present in the adult specimen, were detected at 1000 mg/kg bw/day group for fetuses (79.5%, 116/146 foetuses examined vs 62.1%, 95/153 foetuses examined in controls) but not for litters (23/23 (100%) at all dose levels vs 21/23 (91.3%) in controls). These ossification variations were most prominent in the vertebrae (incompletely ossified first cervical vertebral transverse processes, split thoracic vertebral centrum, incompletely ossified and unossified sacral vertebral transverse processes, and unossified caudal vertebral transverse processes), and sternebrae (incompletely ossified fourth sternebra, and unossified fifth or sixth sternebra). These findings were attributed to test substance administration and relevant for CLP.

Teratogenicity study in rabbits (Anonymous 28, 1988c)

No effects observed for development.

Table 98: Main effects potentially relevant for CLP apart from VSD

Study	Dose level	Significant Effect	Maternal toxicity
Preliminary st udy in Wistar Imamichi rats (Anonymous 25, 1988a)	1000 mg/kg bw/day	↑Foetal mortality (19.4% vs 4.8% of controls) ↓ no. of live foetuses (11.6 vs 14.9 of controls)	↓bw [gestation days 9 (5%), 12 (6%) and 18 (6%)]. ↓bw gain (145%, days 6-9). ↓food consumption [(days 6-9 (37%) and 9-12 (30%)].
Teratogenicity study in Wistar Imamichi rats (Anonymous 26, 1988b)	1000 mg/kg bw/day	↑Foetal mortality (20.8% vs 5.5% of controls) due to ↑resorptions ↓26.6% in the degree of ossification (variation) compared to controls	↓bw from gestation day 9. ↓bw gain [(days 6-9 (150%) and days 12-15 (21%)].
Teratogenicity study in Sprague Dawley rats (Anonymous 27, 1996)	1000 mg/kg bw/day	↑Ossification variations (retarded ossification), or slight ossification irregularities which may or may not be present in the adult specimen for fetuses (79.5%, 116/146 foetuses examined vs 62.1%, 95/153 foetuses examined in controls) but not for litters (23/23 (100%) at all dose levels vs 21/23 (91.3%) in controls).	Anogenital stains (25% vs 4% in controls) and alopecia (16% vs 0% in controls). ↓bw from gestation day 9 (6% on day 9 and 12, 7% on day 16 and 6% on day 20). ↓bw gain on days 6-9 (125%). ↓food consumption days 6-9 (20%) and days 9-12 (13%) in high dose dams though on days 16-20 there was an increase (13%). ↑relative wt of liver (10%).

According to the Dossier Submitter (DS) opinion, the pattern of effects (feotal mortality and ossification varations in rats) displayed in Table 98 is not sufficient for a classification for development according to CLP.

- The effects were observed in developmental studies in rats at 1000 mg/kg bw/day which corresponds to the maximum tolerated dose level (MTD) according to the OECD TG 414.
- Not seen in presence of other relevant variations/malformations or clear signs of developmental toxicity.
- No developmental effects were seen in generational studies in rats or in a teratogenicity study in rabbits.
- Although it is not possible to set an univocal link between maternal toxicity and developmental findings included in Table 98, it cannot be totally discarded a direct consequence considering the remarkable bodyeight gains seen on days 6-9 of the study.

VSD

The two teratogenicity studies performed in rats were assayed in two different strains, Wistar-Imamichi rats and Sprague-Dawley (CD-Crl :(SD) BR) rats. Wistar-Imamichi (WI) rats were generated as an original sub-strain of Wistar rat by Imamichi Institute for Animal Reproduction in Japan. This Institute has routinely used Wistar-Imamichi rat for its reproduction and developmental toxicity studies. On the other hand, the Sprague-Dawley (SD) rat is an outbred rat that was generated by Robert S. Dawley during the 1920s by breeding Wistar rats to hybrids of laboratory derived and wild stocks. Then, in 1950, Charles River Laboratories acquired a breeding stock and developed an own line with better microbial status by caesarian derivation. Afterwards, The Harlan laboratory purchased the Sprague Dawley Company in the 1980s and has maintained its own Sprague-Dawley stock. Although both Charles River and Harlan Sprague-Dawley rats were developed from the original SD company stock, the genetic quality of the populations may have drifted over time due to difference in the breeding practices[10].

In the main teratogenic study performed in Wistar-Imamichi rats (Anonymous 26, 1988b), an increase in ventricular septal defect (VSD) incidences in foetuses was observed at 300 and 1000 mg/kg bw/day dose groups (4% and 6.6%, respectively). VSD is considered a relevant malformation that has been taken into account in the classification of substances such as flumioxacin (RAC opinion, 15 March 2019). In humans, ventricular septal defect is a common congenital heart disease that occurs in almost 50% of all patients with a congenital heart disease. The prevalence is around 4% on asymptomatic infants and the incidence has been estimated between 3.6 and 5.5 cases per 1000 live births (Turner S. W. *et a*l, 2002). Thus, this finding generated a reasonable concern about a potential cardiovascular teratogenic effect of flazasulfuron. The values of VSD obtained in the medium and high dose groups were within the normal foetal incidence range when compared with the 1975-1982 historical control data (range 0-11.3%; mean incidence= 1.47%). This comparison should be taken with caution due to these historical control data exceed the reasonable amount of ± 2 years prior to the study being interpreted (OECD Guidance document on mammalian reproductive toxicity testing and assessment no.43). However, the VSD foetal incidence at 1000 mg/kg bw/day dose group is slightly higher of the 1983-1986 historical control data (range 0-5.3%; mean incidence= 1.52%). Moreover, a VSD foetal incidence of 1.3% was detected in controls. Similar results were obtained in the preliminary study in WI rats (mean foetal incidence= 1.7%, 0% and 6.8% for 0, 50 and 1000 mg/kg bw/day dose groups, respectively) (Anonymous 25, 1988a). The occurrence of the lesion in the main study at 1000 mg/kg bw/day was seen with maternal toxicity (bodyweight reductions and marked decreases in bodyweight and food consumption), whereas in the mid dose group, VSD maternal toxicity was manifested during organogenesis (gestation days 6-9) with decreases in bodyweight gain (50%) and food consumption (11%). In our opinion the occurrence of VSD in Wistar Imamichi rats cannot be univocally linked to maternal toxicity manifested by effects such as those observed in the teratogenicity studies with this strain of rat.

On the other hand, neither in Sprague-Dawley rat strain, nor in New Zealand White rabbit teratogenicity studies, any sign of VSD were detected at any dose level.

Thus, the particular incidence of VSD described in Wistar-Imamichi rat strain must be further evaluated. Several datasets have showed that VSD are common events observed in this rat strain and are considered a transient alteration seen during the course of normal development. Different studies in control Wistar-

[10] Brower M, Grace M, Kotz CM, Koya V. (2015). Comparative analysis of growth characteristics of Sprague-Dawley rats obtained from different sources. Lab. Anim. Res. **31**(4), 166-173.

Imamichi rats displayed VSD incidences close to the mean value recorded at 300 mg/kg bw/day dose group, and slightly lower than detected at 1000 mg/kg bw/day dose group. Aside from the HCD from 1975-1982 and 1983-1986 periods previously addressed, more studies such as summary report in which 1196 foetuses were analysed, presented a VSD incidence of 3.01%, whereas a retrospective study of control animals used in teratogenicity experiments between 1981 and 1984 recorded a mean value of 2.9% at 21 days of gestation (Anonymous 30, 1984). Another control group belonging to a teratogenicity study with ephedrine showed a VSD incidence of 3.2% (Anonymous 31, 1987). In addition, even higher incidence of VSD was observed in adult commercially available Wistar-Kyoto (WKY) rat strain (mean incidence= 22%; range 15-40%; Anonymous 33, 2002).

Interestingly, and considering uniquely Wistar-Imamichi rat strain, a comparison between the VSD incidences reported by Imamichi Institute for Animal Reproduction in Japan and Charles River Company, revealed 20-fold higher VSD incidences in the rats from Imamichi Institute. In addition, if we compared the VSD spontaneously detected in WI rats from Imamichi Institute with the reported in SD rats by Charles River laboratories, the difference increased even 100-fold higher in the WI rats breeded in the Imamichi Institute. Otherwise, control SD rats showed low VSD incidences. HCD from Imamichi Institute revealed a mean VSD incidence of 0.3% between 1975-1983 period, whereas further studies revealed spontaneous values of 2% (Anonymous 32, 1997) and 0.6% (Anonymous 34, 2004). HCD published by Charles River Company reported a mean of 0.016% (range 0-0.85%) for Marta 1993 review, and 0.03% (range 0-0.6%) for the 2011-2015 period (Anonymous 35, 2019). This observation brings out that these disparities are likely consequence of a different genetic background as a result of in-house breeding procedures carried out by each animal facility, so it is more convincing to analyse the results derived from SD rat strains due to control SD rats showed low background VSD incidences.

On the other hand, the incidence of dilation pelvis and ureter in the 300 and 1000 mg/kg bw/day dose groups (0.7% and 1.6%, respectively) in the main rat teratogenicity study would also suggest that visceral alterations are common features in the WI rat strain. These visceral variations were also detected in the 1975-1982 HCD (2% of incidence, range 0-5.6% for renal pelvis dilation and 6.1%, range 0-25.2% for hydroureter, respectively) (Anonymous 29, 1983), and in a summary report of 2444 foetuses (1.35% and 5.48% for renal pelvis dilation and hydroureter incidences, respectively) (Anonymous 30, 1984).

However, a key finding is that the VSD detected on foetuses at gestation day 21 or in early prenatal stages, were progressively reduced during the following postnatal days. A study conducted in breast-fed Wistar-Imamichi rats showed that VSD incidences decrease from 7.6% at day 20 gestation, to 3.01% at day 21 of gestation and no VSD were detected at day 1 of postnatal age or older (Anonymous 30, 1984). Other study conducted in SD rats reported a reduction of 2% to 0% through gestation day 21 to postnatal day 21 (Anonymous 32, 1997).

Similar results were obtained in the same SD strain after trimethadione treatment. In this case, VSD incidences on foetuses on gestation day 21 were 0.6%, 7.6% and 39.6% in the control, low and high dose groups, respectively. Additional foetal examinations on postnatal day 21 resulted in VSD incidences of 0.03%, 0.0% and 6.4% in control, low and high dose groups, respectively (Anonymous 34, 2004). These findings indicate that the VSD found were in the process of closure, which is in concordance to the situation described for humans, where the size of VSD decreases with age.

On the other hand, there were relevant results that showed that VSD incidences were significantly higher compared with control group after cardiovascular-teratogen treatments. For example, ephedrine-treatment generated 27.2% of VSD anomalies at 50 mg/kg bw/day dose level at gestation day 20, compared with 3.2% in controls in WI rats. In two different teratogenic assays, trimethadione treatment in SD rats displayed a mean incidence of 93% and 39.6%, respectively at gestation day 21 in the high dose groups, compared with the incidence of 2% and 0.6% in their respective control groups (Anonymous 31, 1987 and Anonymous 34, 2004, respectively).

It is therefore concluded that ventricular septal defects are visceral variations commonly observed in the Wistar-Imamichi rat strain. The WI rat strain derived from Imamichi Institute for Animal reproduction in Japan presented a VSD average higher than same rat strain breeded in other laboratories, or Sprague-Dawley rat strain. Besides, VSD are considered a transient alteration that tends to disappear during postnatal development,

and the incidence observed in teratogenicity study in rats is so much lower than expected for a cardiovascular teratogen. Thus, the weight of evidence (WoE) indicates that flazasulfuron does not present adverse effects on development of the offspring in the absence of maternal toxicity.

Consequently, classification according CLP regulation is not warranted.

10.10.7Adverse effects on or via lactation

Table 99: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No data			

Table 100: Summary table of human data on effects on or via lactation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 101: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
A single generation reproduction study in rats	Flazasulfuron Code name: SL-160 technical	**F_1 offspring** - (↓) bw at lactation day 14 (7%) and 21 (10%) at 10000 ppm dose group compared with control group.	-It is considered that the effects on pup weight on days 14 and 21 were a direct effect of consumption of the treated diet. -Lactation indices were not affected by treatment.	Anonymous 23 (1993) B.6.6.1.1 (CA)
A two-generation reproduction study in rats	Flazasulfuron Code name: SL-160 technical	**F_1 offspring** - (↓) bw at lactation day 7 (10%), 14 (15%) and 21 (18%) at 10000 ppm dose group compared with control group. **F_2 offspring** - (↓) bw at day 0 (8%), 7 (14%), 14 (15%) and 21 (20%) at 10000 ppm dose group compared with control group.	-Decreased bodyweights observed in F_1 litters were considered a direct effect of consumption of the treated diet. -Lower bodyweights at birth and throughout lactation period, observed in F_2 litters, were considered to be related to reduced maternal weight. -Lactation indices were not affected by treatment.	Anonymous 24 (1995b) B.6.6.1.2 (CA)

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

The available information on the potential of flazasulfuron to cause adverse effects on the offspring via lactation or on lactation is contained in the generation reproductive studies (Anonymous 23, 1993; B.6.6.1.1 and Anonymous 24, 1995b; B.6.6.1.2).

In the generational studies there is no clear evidence of adverse effects in the offspring due to transfer of test substance in the milk, or of adverse effect on the quality of the milk. Only decreased offspring bodyweights were observed on lactation period, but these were considered a direct effect of the solid diet or were related to maternal toxicity.

10.10.9 Comparison with the CLP criteria

Adverse effects on or via lactation are included under reproductive toxicity (described in *section 10.10.3*). The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of classification for sexual function and fertility or developmental toxicity of the substance. This classification can be assigned on the:

-Human evidence indicating a hazard to babies during the lactation period.

-Results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk.

-Absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

No human information is available on the effects of flazasulfuron on or via lactation, but there is information reliable from a single and two-generation reproduction studies in rats.

Based on the data available, there were no effects to warrant classification of flazasulfuron for effects on or *via* lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

There is convincing evidence to conclude that flazasulfuron does not present a reproductive toxicity hazard to humans, therefore, based on the criteria for classification of CLP Regulation (EC) No. 1272/2008, classification as reproductive toxicant is not warranted.

10.11 Specific target organ toxicity-single exposure

Table 102: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
A Range-Finding Acute Neurotoxicity Study in Rats with SL-160 Non-guideline study GLP: Yes (except that	Purity: 95.2-96.6% Oral (gavage): test item suspended in 0.5% w/v methylcellulose Doses: 250, 500, 1000, 2000 mg/kg bw/day. 4-day observation	*Statistical analysis was not performed* **Mortality:** not occurred. **Clinical signs:** The incidence of cage observations through 24 hours after treatment of drooping and/or closed eyelids was slightly higher in the observed treated groups compared with the controls, but a clear dose relationship was not observed and the relation to treatment was equivocal. **Palpebral closure (cage observations)** Dose (mg/kg bw): 0 | 250 | 500 | 1000 | 2000	Anonymous 36 (2002a) (CA) B.6.7.1.1.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**

the periodic analysis of water was not specifically performed under Good Laboratory Regulations)

Rat strain: Charles River Sprague Dawley

3 rats/sex/dose

Study acceptable as a preliminary study

Guideline value for classification: ≤ 2000

mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw

(STOT SE 1)

period

Parameters observed: mortality, clinical signs, bodyweight and neurobehavioural observations

T (h)	3♂	3♀	3♂	3♀	3♂	3♀	3♂	3♀	3♂	3♀
1	1/1/1	4/1/1	1/1/1	1/1/1	1/4/1	1/1/1	1/1/1	1/1/4	4/1/1	1/1/2
2	1/1/1	1/1/1	1/2/1	1/1/2	1/3/1	1/1/1	2/3/1	1/3/1	4/2/2	3/1/4
3	1/1/1	1/1/1	3/1/1	1/1/1	1/2/2	1/1/1	1/1/3	1/3/4	1/1/2	1/1/3
4	1/1/1	1/1/1	1/1/1	1/1/1	1/3/1	1/1/1	2/4/1	1/4/1	4/1/3	4/1/3
5	1/1/1	1/1/1	2/1/1	3/1/1	1/1/1	1/1/1	4/2/3	1/4/1	1/3/2	1/1/3
6	1/1/1	1/1/1	4/1/1	1/1/4	1/3/1	1/4/1	1/1/1	1/1/1	2/1/2	3/1/4
7	1/1/1	1/1/1	2/1/1	1/1/3	1/2/3	1/1/3	1/1/1	1/3/4	3/1/2	1/1/4
8	1/1/1	4/3/1	2/1/2	1/1/1	2/2/2	1/3/1	1/1/2	1/3/4	1/1/1	1/1/4
24	1/1/1	1/1/1	1/1/1	1/1/1	1/2/1	1/1/2	1/1/1	1/1/1	4/1/1	1/1/1

1 = Eyelids wide open
2 = Eyelids slightly drooping (less than half-closed)
3 = Eyelids drooping (greater than or equal to half-closed)
4 = Eyelids completely shut

Posture (cage observations)

	Dose (mg/kg bw)									
	0		250		500		1000		2000	
T (h)	3♂	3♀	3♂	3♀	3♂	3♀	3♂	3♀	3♂	3♀
1	1/1/1	2/1/1	1/1/1	1/1/1	1/2/1	1/1/1	1/1/1	1/1/2	1/1/1	1/1/1
2	1/1/1	4/4/1	1/1/1	1/1/2	1/1/1	1/1/1	1/1/1	1/2/1	2/1/1	2/1/2
3	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/2/2	1/1/1	1/1/2
4	1/1/1	4/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/2/1	1/2/1	2/1/1	2/1/2
5	1/1/1	1/1/1	1/1/1	2/1/1	1/1/1	1/1/1	2/1/1	1/4/2	1/1/1	1/1/2
6	1/1/1	1/1/1	2/1/1	1/1/2	1/1/1	1/2/1	1/1/1	1/1/1	1/1/1	2/1/2
7	1/1/1	1/1/1	1/1/1	1/1/2	1/1/1	1/1/2	1/1/1	1/2/2	1/1/1	1/1/2
8	1/1/1	2/2/1	1/1/1	1/1/1	1/1/1	1/2/1	1/1/1	1/2/2	1/1/1	1/1/2
24	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	1/1/1	2/1/1	1/1/1

1 = Awake, sitting, lying or standing normally
2 - Asleep, lying on side or curled up
3 = Pacing, constantly moving about cage
4 = Rearing (either supported or unsupported)
5 = Head bobbing or head weaving
6 = Flattened limbs spread out, may be clutching bottom of cage
7 = Crouched over/hunched
8 = Dead

Changes in the appearance of the fur in ♀. Observations of rough, unkempt appearance to soiled coat, discolored fur, urine stains and bare spots were recorded. 1 ♀ in at 250 mg/kg/day and 1 ♀ at 1000 mg/kg/day had soiled coats from the first and second hour, respectively, through 24 hours. Beginning at the 5-hour interval, 1 ♀ at 500 mg/kg/day and 1 ♀ at 2000 mg/kg/day had changes in the appearance of the fur. At the 6-hour interval another 1000 mg/kg/day ♀ and an additional 2000 mg/kg/day ♀ had changes in the appearance of the fur. These observations were present at each subsequent interval through 24 hours. No other changes in observations from control animals were observed during handling.

1 ♂ at 2000 mg/kg/day had an arousal rating of low beginning at the 2-hour interval and lasting through the 5-hour interval. 1 ♂ at 1000 mg/kg/day had an arousal rating of low beginning at the 3-hour interval and lasting through the 8-hour interval. 1 ♂ at 500 mg/kg/day had an arousal rating of low begirming at the 5-hour interval and lasting through the 7-hour interval, and 1 ♂ at 250 mg/kg/day had an arousal rating of low beginning at the 3-hour interval and lasting through the 7-hour interval. All males were alert at 24 hours.

1 ♀ at 2000 mg/kg/day female had an arousal rating of low at the 4-, 7- and 8-hour intervals. A hunched body posture was recorded for this animal at the 8-hour interval. 1 ♀ at 1000 mg/kg/day had an arousal rating of low at the 5- and 6-hour intervals. All ♀ were alert at 24 hours.

Bodyweight: (↓ ns) bw and bw gain in ♂ (12%/55%) at 2500 mg/kg/day

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		NOAEL: not derived since it is a preliminar study.	
An Acute Neurotoxicity Screening Study in Rats with SL-160 US EPA FIFRA 81-8 GLP: Yes (except that the periodic analysis of water was not specifically performed under Good Laboratory Regulations) Rat strain: Charles River Sprague Dawley 10 rats/sex/dose **Study acceptable** *Guideline value for classification: ≤ 2000* *mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw* *(STOT SE 1)*	Purity: 95.3 Oral (gavage): test item suspended in 0.5% w/v methylcellulose (aq) Doses: 50, 1000, 2000 mg/kg bw/day. 14-day observation period Parameters observed: mortality, clinical signs, bodyweight neuropathology and neurobehavioural assessment.	**Mortality:** not occurred. **Clinical signs:** One ♀ of the control group had decreased feces on Day 3. One ♀ in 1000 mg/kg/day group had anogenital staining on Day 1. Another 1000 mg/kg/day ♀ had decreased feces and anogenital staining on Days 1 through 4, dried red material around both eyes on Day 1, and dried red material around mouth and both forepaws on Days 2 and 3. It was also noted that this ♀ appeared thin on Days 2 through 4. Two 2000 mg/kg/day ♀ had anogenital staining, one on Day 1 and the other on Days 1 through 4. Decreased feces were also observed on Days 1 and 2 for the 2000 mg/kg/day ♀ that had anogenital staining on Day 1. The toxicological significance of these findings is equivocal. Mean motor activity measurements for 1000 and 2000 mg/kg bw/day dose in ♂ and 2000 mg/kg bw/day in ♀ were statistically significantly different from the respective control groups on Day 0 (5 hours postdosing). Animals were less active with more resting time than controls. In ♂, on day 0, at the doses of 1000 and 2000 mg/kg/day, changes in distance travelled (↓51%/↓59%), resting time (↑26%/↑35%), ambulatory time (↓50%/↓60%), stereotypic time (↓34%/↓49%), bursts of stereotypic (↓41%/↓53%), horizontal counts (↓47%/↓59%), total counts (↓54%/↓62%), vertical count (↓76%/↓77%). In ♀, on day 0, at the dose of 2000 mg/kg/day changes in distance travelled (↓58%), resting time (↑28%), ambulatory time (↓54%), stereotypic time (↓32%) and bursts of stereotypic (↓37%); and at the doses of 1000 and 2000 mg/kg/day in horizontal counts (↓36%/↓56%), total counts (↓40%/↓64%), vertical count (↓55%/↓80%). The effect, which was considered an indication of generalised toxicity probably due to test system administration (bolus) and not due to neurotoxicity, was reversed by Day 7. **Bodyweight**: no effects. **Necropsy**: cervical lymph nodes enlarged in ♂ (1/5) at the dose of 1000 mg/kg/day and in ♀ at 50 (2/5) and 1000 (1/5) mg/kg bw/day. Both uterine horns enlarged/filled with clear fluid at 1000 (1/5) and 2000 (1/5) mg/kg bw/day. **$NOAEL_{Systemic}$: 2000 mg/kg bw (highest dose tested)** **$NOAEL_{Neurotoxicity}$: 2000 mg/kg bw (highest dose tested)**	Anonymous 37 (2002b) (CA) B.6.7.1.2.

Table 103: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 104: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. Relevant information for STOT SE is covered by acute toxicity studies in form of clinical observations, and macroscopic and microscopic pathological examination that can reveal hazards that may not be life-threatening but could indicate functional impairment. No findings were observed in acute toxicity studies in rats (Anonymous 11, 1988a) and mice (Anonymous 12, 1988b) inlucded in *section 10.1*. Information from acute neurotoxicity studies is available in Table 102.

STOT SE 3

STOT SE 3 includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2.

According to the results of the acute inhalation studies, respiratory tract irritation was not observed after administration of flazasulfuron.

Narcotic effects were not observed in acute toxicity studies.

STOT SE 1 and 2

STOT-SE Category 1 and 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement. No effects were observed in acute toxicity studies.

10.11.2 Comparison with the CLP criteria

No effects were observed for STOT SE 1 (guidance value for classification: ≤ 300 mg/kg bw).

The only effects observed in the range for STOT SE 2 after oral administration included in Table 102 are not relevant for classification (guidance value for classification: ≤ 2000 mg/kg bw and >300 mg/kg bw):

In the acute neurotoxicity study (Anonymous 37, 2002b) alterations in mean motor activity measurements at the doses of 1000 and 2000 mg/kg bw/day in ♂ and 2000 mg/kg bw/day in ♀ were statistically significantly different from the respective control groups on day 0 (5 hours postdosing). Animals were less active with more resting time than controls. No changes on subsequent days were observed. The effect was considered probably due to test system administration (bolus) and not associated to neurotoxicity.

It has to be noted that in the available rat carcinogenicity study (Anonymous 20, 1995a) decreased activity was seen in 42/50 animals (84%) on week 66-93 vs 6/50 in controls mainly observed only in one week/animal but in 4 animals with 3-5 weeks of duration. These effects are out of the scope of the STOT SE regarding the onset on week 66-93.

No relevant effects were observed after dermal application or inhalation of flazasulfuron.

No signs were observed to be regarded for classification for STOT SE 3 according to CLP Regulation (respiratory tract irritation and narcotic effects).

10.11.3 Conclusion on classification and labelling for STOT SE

Flazasulfuron does not require classification for STOT SE according to CLP Regulation.

10.12 Specific target organ toxicity-repeated exposure

Table 105: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
6 week feeding study in mice with SL-160 Method: 92/69/EEC part B7 Guideline GLP: Yes Deviations from current test guideline (OECD TG 453, 2018): In-live portion lasted 6 weeks rather than 28 days. Mice strain: Charles River Crl:CD-1 (ICR) BR VAF/PLUS mice 10 mice/sex/dose **Study acceptable** Guideline value for classification extrapolated to 6-week study: *STOT RE 1 ≤ 21.4 mg/kg bw/day* *STOT RE 2 ≤ 214.3 mg/kg bw/day*	Purity: 97.3% Oral (diet). Doses: 200, 1000, 5000, 10000 ppm, equivalent to 34, 181, 884, 1745 mg/kg/day (m) and 43, 212, 1032, 2036 mg/kg/day (f). daily for 6 weeks. Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, biochemistry, organ weights, gross pathology and histopathology	Mortality: No mortality was observed. 10000 ppm (1745 ♂/2036 ♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> (↓) bw in ♂ [week 4 (7% ndr)] and in ♀ [week 1 (7%), week 2 (7%), week 3 (7%), week 4 (8%), week 6 (7% ncdr)] (↓) terminal bw in ♂ (8%) and in ♀ (11%) (↓) bwg in ♂ [week 1 (100%) week 2 (62%), week 3 (50%), week 4 (58%), week 5 (36%), week 6 (26% ns)] (↓) abs food consumption in ♂ [week 5 (7%)] <u>Haematology:</u> (↓) platelet count in ♂ (19%) <u>Clinical chemistry:</u> (↑) cholesterol in ♂ (24% ns, ncdr) (↑) albumin in ♂(10%) (↑) A/G ratio in ♂ (17%) and ♀ (26%) <u>Organ weights:</u> Liver: (↑) abs weight in ♂ (23%) and ♀ (20%) Liver: (↑) rel weight in ♂ (33%) and ♀ (35%) <u>Histopathology:</u> Liver: centrilobular hepatocyte hypertrophy in 10/10 ♂ and 10/10 ♀ 5000 ppm (884 ♂/1032 ♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> (↓) bw in ♀ [week 6 (7% ncdr)] <u>Clinical chemistry:</u> (↑) cholesterol in ♂ (31% ncdr) <u>Organ weights:</u> Liver: (↑) abs weight in ♂ (12%) Liver: (↑) rel weight in ♂ (13%) and ♀ (15%) <u>Histopathology:</u> Liver: centrilobular hepatocyte hypertrophy in 9/10 ♂ and 2/10 ♀ **NOAEL 1000 ppm, [181 mg/kg bw/day (♂) and 212 mg/kg bw/day (♀),]** for ♂ and ♀ based on centrilobular hepatocyte hypertrophy and (↑)abs and rel liver weight at 5000 ppm (LOAEL).	Anonymous 38 (1992) (CA) B.6.3.1.1
SL-160 Technical: 4-week dose range finding study in rats Method: 92/69/EEC part B7 Guideline GLP: Yes Deviations from current test guideline: only liver and kidneys were examined microscopicaly Rat strain: Charles River Fischer 344 (DuCrj)	Purity: 96.3% Oral (diet). Doses: 100, 1000, 5000, 10000 and 20000 ppm equivalent to 7.5, 72.2, 354, 731 and 1145 mg/kg/day (m) and 8.9, 88.0, 424, 834 and 1544 mg/kg/day (f). Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, biochemistry,	Mortality: No mortality was observed. 20000 ppm (1145 ♂/1544 ♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> (↓) abs bw in ♂ [week 1 (30%), week 2 (29%), week 3 (27%), week 4 (24%)] and in ♀ [week 1 (26%), week 2 (23%), week 3 (23%), week 4 (21%)] (↓) terminal bw in ♂ (26%) and in ♀ (22%) (↓) food consumption in ♂ (35%) and in ♀ (32%) <u>Haematology:</u> (↓) Hematocrit in ♂ (10% ncdr) and ♀ (14% ncdr) (↓) Hemoglobin in ♂ (9% ncdr) and ♀ (12% ncdr) (↓) Erythrocyte in ♂ (12%) and ♀ (12% ncdr) (↓) platelet count in ♂ (11%) (↓) MCV in ♀ (2%) (↑) MCH in ♂ (3%) and ♀ (%) <u>Clinical chemistry:</u> (↓) ALP in ♂ (34% ncdr) and ♀ (33% ncdr) (↓) GOT in ♂ (23%) (↓) TG in ♂ (42%)	Anonymous 39 (1988a) (CA) B.6.3.1.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
6 rats/sex/dose **Study acceptable** as a rangefinding study Guideline value for classification extrapolated to 4-week study: *STOT RE 1 ≤ 30 mg/kg bw/day* *STOT RE 2 ≤ 300 mg/kg bw/day*	organ weights, urianalysis, gross pathology and histopathology in liver and kidney in two animals each.	(↓) Creatinine in ♂ (8% ns, ndr) (↓) Glucose in ♂ (13%) (↑) TP in ♂ (10% ncdr) and ♀ (6%) (↑) Alb in ♂ (13% ncdr) and ♀ (25%) (↑) Glob in ♂ (6% ndr) (↑) K in ♂ (5% ndr) and ♀ (13%) (↑) Ca in ♂ (8% ncdr) and ♀ (4%) (↑) P in ♀ (17%) (↑) A/G ratio in ♂ (6%) and ♀ (6%) (↑) T chol in ♂ (110%) and ♀ (64% ncdr) (↑) GGTP in ♂ (76%) and ♀ (133% ndr) (↑) T bil in ♀ (600%) <u>Urianalysis:</u> (↓) Specific gravity in ♂ (4%) (↓) protein in ♂ (6/6) <u>Organ weights:</u> Liver: (↑) abs wt in ♂/♀ (26% ncdr/32%) and (↑) rel wt in ♂/♀ (71% ncdr/68%) Kidney: (↓) abs wt in ♂/♀ (8% ns, ndr) (↑) rel wt in ♂/♀ (27% ncdr/23% ncdr) Brain: (↓) abs wt in ♂/♀ (6% ndr/36%) and (↑) rel wt in ♂/♀ (28%/24% ncdr) Pituitary: (↓) abs wt in ♂/♀(23%/31%) Thyroid: (↑) rel wt in ♂/♀ (39% ncdr/29%) Heart: (↓) abs wt in ♂/♀ (21%/12%) and (↑) rel wt in ♂/♀(20%/11% ncdr) Thymus: (↓) abs wt in ♂/♀ (18%/13%) Spleen: (↓) abs wt in ♂/♀ (23% ncdr/19%) Adrenal: (↓) abs wt in ♀ (19%) and (↑) rel wt in ♂/♀ (29%) Testes: (↓) abs wt in ♂(5%) and (↑) rel wt in ♂(27%) <u>Macroscopic pathology:</u> Liver: ▪ enlargement ♂ (5/6) and ♀ (5/6). ▪ (ns) centrilobular hepatocyte swelling in ♂ (2/2) and ♀ (2/2). 10000 ppm (731 ♂/834 ♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> (↓) abs bw in ♂ [week 1 (16%), week 2 (12%), week 3 (11%), week 4 (7%)] and in ♀ [week 1 (12%), week 2 (10%), week 3 (10%), week 4 (8%)] (↓) terminal bw in ♂ (9%) and in ♀ (8%) <u>Haematology:</u> (↓) Hematocrit in ♂ (5% ncdr) and ♀ (5% ncdr) (↓) Hemoglobin in ♂ (5% ncdr) and ♀ (5% ncdr) (↓) Erythrocyte in ♀ (5% ncdr) <u>Clinical chemistry:</u> (↓) ALP in ♂ (19% ncdr) and ♀ (15% ncdr) (↓) TG in ♂ (32%) (↑) TP in ♂ (6% ncdr) and ♀ (4%) (↑) Alb in ♂ (8% ncdr) and ♀ (7%) (↑) Ca in ♂ (3% ncdr) and ♀ (3%) (↑) A/G ratio in ♂ (4%) (↑) T chol in ♀ (10% ncdr) <u>Organ weights:</u> Liver: (↑) abs wt in ♂/♀ (19% ncdr/32%) and (↑) rel wt in ♂/♀ (30% ncdr/68%) Kidney: (↑) rel wt in ♂/♀ (23% ncdr/23% ncdr) Brain: (↓) abs wt in ♀ (30%) and (↑) rel wt in ♂/♀ (8%/6% ncdr)	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
		Thyroid: (↑) rel wt in ♂ (43% ncdr) Heart: (↑) rel wt in ♂/♀(12%/11% ncdr) Spleen: (↓) abs wt in ♂ (8% ncdr) Adrenal: (↑) rel wt in ♂ (24%) Testes: (↑) rel wt in ♂(9%) Histopathology: Liver: ▪ (ns) centrilobular hepatocyte swelling in ♂ (2/2). 5000 ppm (354 ♂/424 ♀ mg/kg bw/day) Bodyweight and food consumption: (↓) abs bw in ♂ [week 1 (8%), week 2 (6%), week 3 (6%), week 4 (6%)] and in ♀ [week 2 (5%), week 3 (7%), week 4 (8%)] (↓) terminal bw in ♂ (7%) and in ♀ (7%) Haematology: (↓) Hematocrit in ♂ (7% ncdr) and ♀ (5% ncdr) (↓) Hemoglobin in ♂ (5% ncdr) and ♀ (5% ncdr) (↓) Erythrocyte in ♀ (5% ncdr) Clinical chemistry: (↓) ALP in ♂ (31% ncdr) and ♀ (22% ncdr) (↓) TG in ♂ (28%) (↑) TP in ♂ (7% ncdr) and ♀ (3%) (↑) Alb in ♂ (8% ncdr) (↑) Ca in ♂ (3% ncdr) (↑) T chol in ♀ (21% ncdr) Organ weights: Liver: (↑) abs wt in ♂/♀ (27% ncdr/12%) and (↑) rel wt in ♂/♀ (36% ncdr/21%) Kidney: (↑) rel wt in ♂/♀ (25% ncdr/11% ncdr) Brain: (↑) rel wt in ♀ (7% ncdr) Heart: (↑) rel wt in ♂(9%) Spleen: (↓) abs wt in ♂ (9% ncdr) Testes: (↑) rel wt in ♂(7%) 1000 ppm (72.2 ♂/88.0 ♀ mg/kg bw/day) Clinical chemistry: (↓) ALP in ♂ (6%) Organ weights: Liver: (↑) abs wt in ♂ (8% ncdr) and (↑) rel wt in ♂ (8% ncdr) Kidney: (↑) rel wt in ♂ (8% ncdr) **NOAEL 1000 ppm** [equivalent to a dosage of **72.2 mg/kg bw/day (♂) or 88 mg/kg bw/day (♀)**] based on increased liver and kidney weights accompanied by changes in biochemistry at 5000ppm (LOAEL).	
SL-160 technical: 4-Week oral toxicity study in dogs Non-guideline study GLP: Yes Dog strain: Beagle 1 dog/sex/dose Statistics not performed **Preliminary test**	Purity: 97.3% Oral (gelatine capsules). Doses: 10, 100, 500 and 1000 mg/kg/day daily for 4 weeks. Parameters observed: mortality, clinical signs, bodyweight	*Since statistical analysis was not performed only dose-dependent effects are included in the table*. Mortality: Both animals of the 1000 mg/kg/day group were terminated in extremis ♂ on day 22/♀ on day 18; ♀ of the 100 mg/kg/day was found dead on day 22. 1000 mg/kg/day Clinical signs: Vomiting of foamy fluid, food or test substance was observed in each treatment group. ♂ decreased spontaneous motor activity and unsteady standing posture, petechia or hemorrhage in the oral cavity, conjunctival	Anonymous 40 (1992) (CA) B.6.3.1.3

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
Guideline value for classification extrapolated to 4-week study: STOT RE 1 ≤ 30 mg/kg bw/day STOT RE 2 ≤ 300 mg/kg bw/day	and food intake, haematology, biochemistry, organ weights, urianalysis, ophthalmology, gross pathology and histopathology.	congestion in the eyes and swelling in the left hindleg. ♀ lateral recumbent posture and bloody stool. Decreased spontaneous motor activity, petechia or hemorrhage in the oral cavity, conjunctival congestion in the eyes and intussusception of the intestine. <u>Bodyweight and food consumption:</u> (↓ ns) terminal bw in ♂ (26%) and in ♀ (25%) (↓ ns) food consumption in ♂ (38%) and in ♀ (46%) <u>Haematology:</u> week 2 due to the death of the animals (↓ ns) Hematocrit in ♂ (21% , ndr) and ♀ (25%) (↓ ns) Hemoglobin in ♂ (19%, ndr) and ♀ (24%) (↓ ns) Erythrocyte in ♂ (22%, ndr) and ♀ (25%) (↓ ns) platelet count in ♂ (96%, ndr) and ♀ (96%) (↓ ns, ndr) WBC in ♂ (53%) and ♀ (65%) (↓ ns) Lymphocyte in ♂ (55%) and ♀ (56%) (↓ ns, ndr) Neutrophil in ♂ (55%) and ♀ (88%) (↑ ns, ndr) Monocyte in ♂ (500%) and ♀ (300%) (↓ ns, ncdr) Eosinophil in ♂ (100%) and ♀ (100%) <u>Clinical chemistry:</u> week 2 due to the death of the animals (↑ ns) ALP in ♂ (529%) and ♀ (218%) (↑ ns) GOT in ♂ (48%) and ♀ (39%) (↑ ns) GPT in ♂ (265%) and ♀ (314%) (↓ ns) CPK in ♀ (44% ndr) (↓ ns) TP in ♀ (20%) (↓ ns) Creatinine in ♂ (54%, ncdr) and ♀ (37%) (↓ ns) BUN in ♂ (42%, ndr) and ♀ (8%, ncdr) (↓ ns) Glucose in ♂ (15%) and ♀ (19%) (↑ ns) TG in ♂ (133% ndr) and ♀ (70% ncdr) (↓ ns) Alb in ♂ (24%) and ♀ (39%) (↑ ns) Glob in ♂ (26%) (↓ ns) K in ♂ (10%) (↓ ns) Ca in ♂ (14%, ncdr) and ♀ (20%) (↓ ns) P in ♂ (79%) and ♀ (51%) (↓ ns) A/G ratio in ♂ (40%) and ♀ (45%) (↓ ns) T chol in ♂ (7%) and ♀ (12%) (↑ ns) GGTP in ♂ (150%) and ♀ (100%) (↑ ns) T bil in ♂ (861%) and ♀ (319%) <u>Urianalysis:</u> (↑ ns) Occult blood in ♂ (↑ns) Bilirrubin in ♂ (↑ ns) RBC in ♂ <u>Organ weights:</u> Terminal organ weights were not recorded in the animals of this dose. <u>Macroscopic pathology:</u> Subcutaneous tissue: ▪ Yellow in colour in ♂ (1/1 ndr) and in ♀ (1/1) ▪ Hemorrhage/petechial in ♂ (1/1) and in ♀ (1/1) ▪ Abscess in ♂ (1/1) ▪ Edema in ♂ (1/1)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		Eye: ▪ Conjunctival congestion in ♀ (1/1) Heart: ▪ Petechia in ♀ (1/1 ndr) ▪ Hydrocardia in ♀ (1/1) Tongue: ▪ Ulcer in ♂ (1/1) Oral cavity: ▪ Petechia in ♀ (1/1 Stomach: ▪ Red spot/Petechia in ♂ (1/1) Intestine: ▪ Red in color/Petechia in ♂ (1/1) ▪ Intussusception in ♀ (1/1) Liver: ▪ Swelling in ♂ (1/1) and in ♀ (1/1) ▪ Yellow in colour in ♀ (1/1 ndr) Gall bladder: ▪ Distended with bile in ♂ (1/1) Kidney: ▪ Swelling in ♀ (1/1) Spleen: ▪ Swelling in ♂ (1/1) Urinary bladder: ▪ Red spot/Petechia in ♂ (1/1) Testis: ▪ Atrophy in ♂ (1/1) Prostate: ▪ Atrophy in ♂ (1/1) Epididymis: ▪ Red spot in ♂ (1/1) Lymph nodes: ▪ Red in colour in ♀ (1/1 ndr) ▪ Swelling in ♂ (1/1) <u>Histopathology:</u> Liver ▪ Difuse hepatocellular swelling in ♂ (1/1) and in ♀ (1/1) ▪ Hepatitis in ♀ (1/1 ndr) ▪ Increased deposition of brown pigment in ♂ (1/1) and in ♀ (1/1) Kidney ▪ Degeneration/necrosis of tubular cells in ♂ (1/1) Adrenal: ▪ Cortical hyperplasia in ♂ (1/1) and in ♀ (1/1) Spleen: ▪ Congestion in ♀ (1/1) ▪ Hemorrhage in ♂ (1/1) Bone marrow: ▪ Increased hematopoiesis in ♀ (1/1 ndr) Thymus: ▪ Atrophy in ♂ (1/1) and in ♀ (1/1 ndr) Intestine: ▪ Intussusception in ♀ (1/1) Heart: ▪ Endocarditis in ♀ (1/1) ▪ Hemorrhage in ♀ (1/1) Lymph nodes: ▪ Abscess in ♂ (1/1) and in ♀ (1/1	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		▪ Hemorrhage in ♀ (1/1) Subcutaneous tissue: ▪ Abscess in ♂ (1/1) ▪ Hemorrhage in ♂ (1/1) Tongue: ▪ Ulcer in ♂ (1/1) Prostate: ▪ Atrophy in ♂ (1/1) Eye: ▪ Hemorrhage in ♀ (1/1) Epididymis: ▪ Hemorrhage in ♂ (1/1) Urinary bladder: ▪ Hemorrhage in ♂ (1/1) 500 mg/kg/day <u>Clinical signs:</u> ♂ raised area in the tongue with bleeding from day 11 to 21 of treatment. Petechia and hemorrhage in the oral cavity and conjunctival congestion in the eyes until week 4. Decreased spontaneous motor activity and swelling or mass in the interdigital region of the right foreleg. <u>Bodyweight and food consumption:</u> (↓ ns) terminal bw in ♂ (20%) and in ♀ (14%) (↓ ns) food consumption in ♂ (12%) <u>Haematology:</u> Week 4 (↓ns) Hematocrit in ♂ (44%) and ♀ (18%) (↓ ns) Hemoglobin in ♂ (51%) and ♀ (16%) (↓ ns) Erythrocyte in ♂ (55%) and ♀ (14%) (↓ ns) Lymphocyte in ♂ (56%) (↓ ns) Eosinophil in ♂ (100%) (↑ ns) MCV in ♂ (24%) (↑ ns) MCH in ♂ (9%) (↓ ns) MCHC in ♂ (12%) (↑ ns) retics in ♂ (700%) (↑ ns) WBC in ♀ (28%) (↓ ns) Lymphocyte in ♂ (57%) and ♀ (85%) Week 2 (↓ ns) Lymphocyte in ♂ (40%) (↓ ns, ncdr) Eosinophil in ♂ (100%) (↓ ns) Hematocrit in ♀ (21%) (↓ ns) Hemoglobin in ♀ (19%) (↓ ns) platelet count in ♀ (21%) <u>Clinical chemistry:</u> week 4 (↑ ns) ALP in ♀ (217%) (↑ ns) GOT in ♀ (29%) (↑ ns) GPT in ♂ (25%) and ♀ (262%) (↓ ns) CPK in ♂ (28%) and ♀ (113% ncdr) (↓ ns) TP in ♂ (17%) and ♀ (20% ncdr) (↓ ns) Creatinine in ♂ (48% ncdr) and ♀ (14%) (↓ ns) BUN in ♂ (37% ncdr) (↓ ns) Glucose in ♂ (16%) and ♀ (10%)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		(↑ ns) TG in ♂ (234% ncdr) and ♀ (20% ncdr) (↓ ns) Alb in ♂ (43%) and ♀ (34% ncdr) (↓ ns) Ca in ♂ (13%) and ♀ (11%) (↓ ns) P in ♂ (54%) (↓ ns) A/G ratio in ♂ (51%) and ♀ (33%) (↑ ns) T bil in ♀ (12%) week 2 (↑ ns) ALP in ♂ (111%) and ♀ (18%) (↑ ns) GOT in ♂ (27%) and ♀ (25%) (↓ ns) TP in ♀ (13%) (↓ ns) Creatinine in ♂ (42%, ncdr) and ♀ (14%) (↓ ns) BUN in ♀ (12%, ncdr) (↓ ns) Alb in ♀ (26%) (↓ ns) A/G ratio in ♂ (37%) and ♀ (31%) (↑ ns) T bil in ♂ (28%) (↓ ns) Ca in ♂ (14%, ncdr) and ♀ (6%) (↓ ns) P in ♂ (43%) and ♀ (33%) (↓ ns) K in ♂ (9%) <u>Organ weights:</u> Liver: (↑ ns) abs wt in ♂/♀ (88%/41%) and (↑ ns) rel wt in ♂/♀ (135%/63%) Kidney: (↑ ns) abs wt in ♀ (51%) and (↑ ns) rel wt in ♂/♀ (86%/22%) Brain: (↓ ns) abs wt in ♂(9%) and (↑ ns) ♀ 6%) and (↑ ns) rel wt in ♂/♀ (13%/23%) Pituitary: (↑ ns) abs wt in ♀(16%) and (↑ ns) rel wt in ♂/♀ (36%/35%) Thyroid: (↑ ns) abs wt in ♂ (20%) and (↑ ns) rel wt in ♂/♀ (50%/16%) Heart: (↓ ns) abs wt in ♀ (16%) and (↑ ns) rel wt in ♂ (16%) Pancreas: (↑ ns) abs wt in ♂/♀ (52%/13%) and (↑ ns) rel wt in ♂/♀ (90%/32%) Spleen: (↑ ns) abs wt in ♂(290%) and (↑ ns) rel wt in ♂ (395%) Adrenal: (↑ ns) abs wt in ♀ (48%) and (↑ ns) rel wt in ♂/♀ (25%/67%) Testes: (↓ ns) abs wt in ♂(31%) Prostate: (↓ ns) abs wt in ♂(52%) and (↓ ns) rel wt in ♂(38%) Ovaries: (↓ ns) abs wt in ♀ (52%) and (↓ ns) rel wt in ♀ (23%) <u>Macroscopic pathology:</u> Subcutaneous tissue: ▪ Hemorrhage/petechial in ♂ (1/1) and in ♀ (1/1) ▪ Abscess in ♂ (1/1) Liver: ▪ Swelling in ♂ (1/1) Spleen: ▪ Swelling in ♂ (1/1)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		Urinary bladder: ▪ Red spot/Petechia in ♂ (1/1) <u>Histopathology:</u> Liver ▪ Difuse hepatocellular swelling in ♂ (1/1) and in ♀ (1/1) Adrenal: ▪ Cortical hyperplasia in ♀ (1/1) Thymus: ▪ Atrophy in ♂ (1/1) Subcutaneous tissue: ▪ Abscess in ♂ (1/1) ▪ Hemorrhage in ♂ (1/1) Urinary bladder: ▪ Hemorrhage in ♂ (1/1) 100 mg/kg/day <u>Hematology:</u> Week 4 (↓ ns) Lymphocyte in ♂ (30%) and ♀ (56%) <u>Clinical signs:</u> ♀ decreased spontaneous motor activity prior to death. <u>Clinical chemistry:</u> Week 4: (↓ ns) Creatinine in ♂ (15% ncdr) (↓ ns) BUN in ♂ (23% ncdr) (↑ ns) TG in ♂ (79% ncdr) Week 2 (↓ ns) Alb in ♀ (6%) (↑ ns) GPT in ♀ (95%) (↓ ns) Ca ♀ (5%) (↓ ns) A/G ratio in ♀ (14%) <u>Organ weights:</u> Organ weights were not measured in the female treated with 100 mg/kg/day Liver: (↑ ns) rel wt in ♂(20%) Thyroid: (↑ ns) abs wt in ♂ (19%) and (↑ ns) rel wt in ♂ (19%) Heart: (↑ ns) rel wt in ♂ (16%) Pancreas: (↑ ns) abs wt in ♂ (21%) and (↑ ns) rel wt in ♂ (29%) Spleen: (↑ ns) abs wt in ♂(11%) and (↑ ns) rel wt in ♂ (18%) <u>Histopathology:</u> Adrenal: ▪ Cortical hyperplasia in ♀ (1/1) 10 mg/kg/day <u>Hematology:</u> Week 4 (↓ ns) Lymphocyte in ♂ (21%) and ♀ (56%) <u>Clinical chemistry:</u>	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
		Week 4: (↓ ns) CPK in ♀ (29% ncdr) (↓ ns) Creatinine in ♂ (17% ncdr) (↓ ns) BUN in ♂ (24% ncdr) (↑ ns) TG in ♂ (83% ncdr) and ♀ (55% ncdr) (↓ ns) Alb in ♀ (6% ncdr) Week 2 (↓ ns) Creatinine in ♂ (16%, ncdr) Week 2 (↓ ns) A/G ratio in ♀ (8%) <u>Organ weights:</u> Liver: (↑ ns) rel wt in ♂(12%) Heart: (↓ ns) abs wt in ♀ (12%) Brain: (↑ ns) rel wt in ♀ (16%) Pancreas: (↑ ns) abs wt in ♀ (9%) and (↑ ns) rel wt in ♀ (24%) Adrenals: (↑ ns) abs wt in ♀ (30%) and (↑ ns) rel wt in ♀ (33%) **NOAEL not derived** since it is a palatability study.with only one animal per sex and dose.	
SL-160 technical: 13-Week oral subchronic toxicity study in rats Method: US EPA FIFRA Guideline No 82-1, comparable to 87/302/EEC part B GLP: Yes Deviations from current test guideline (OECD TG 409, 1998): uterus, epididymis and gall bladder weights were not recorded. Rat strain: SPF Fischer rats (F344/DuCrj) 12 rats/sex/dose **Study acceptable** STOT RE 1 ≤ 10 mg/kg bw/day STOT RE 2 ≤ 100 mg/kg bw/day	Purity: 96.3% Oral (diet). Doses: 40, 200, 1000 and 5000 ppm, equivalent to 2.3, 11.7, 57.1, 287 mg/kg/day (m) and 2.5, 12.8, 61.5, 309 mg/kg/day (f). Daily for 13 weeks. Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, biochemistry, organ weights, urianalysis, ophthalmology, gross pathology and histopathology.	Mortality: 1 ♂ rat of the 1000 ppm group was found dead in week 13 5000 ppm (287 ♂/309 ♀ mg/kg bw/day) <u>Clinical signs:</u> ♀ (1/12) blotted fur on external genital region. <u>Bodyweight and food consumption:</u> (↓) bw in ♂ [week 1 (6%), week 2 (6%), week 3 (5%), week 4 (5%), week 5 (4%), week 6 (4%), week 7 (5%), week 8 (4%),week 9 (5%), week 10 (5%), week 11 (5%), week 12 (5%),week 13 (5%)] and in ♀ [week 1 (5%), week 2 (6%), week 3 (6%), week 4 (8%), week 5 (10%), week 6 (10%), week 7 (9%), week 8 (10%),week 9 (12%), week 10 (14%), week 11 (13%), week 12 (12%),week 13 (13%)] (↓) terminal bodyweight in ♀ (13%) (↓) food consumption in ♂ [week 1 (18%)] and in ♀ [week 1 (28%), week 5 (11%) and week 8 (11%)] <u>Haematology:</u> (↓) Hematocrit in ♂ (4%) (↓) Hemoglobin in ♀ (3%) <u>Clinical chemistry:</u> (↓) ALP in ♂ (14%) (↑) GGTP in ♂ (22%) (↓) Creatinine in ♂ (10%) and ♀ (4%) (↑) T chol in ♂ (15%) and ♀ (10% ncdr) (↓) GPT in ♀ (14% ncdr) (↑) Alb in ♀ (4% ndr) (↑) P in ♀ (20% ncdr) (↓) Cl in ♀ (1% ndr) <u>Urianalysis:</u> (↓) Specific gravity in ♂ (1%) (↑) Urine volume ♂ (44%) <u>Organ weights:</u>	Anonymous 41 (1988b) (CA) B.6.3.2.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
		Liver: (↑) abs weight in ♂ (13%) and ♀ (20%) and rel weight in ♂ (19%) and ♀ (16%) Kidney: (↑) abs weight in ♂ (52%) and ♀ (20%) and (↑) rel weight in ♂ (60%) and ♀ (16%) Brain: (↓) abs weight in ♀ (27%) and (↑) rel weight in ♂ (6%) and ♀ (11%) Pituitary: (↑) rel weight in ♀ (17%) Heart: (↑) rel weight in ♀ (10% ndr) Thyroid: (↑) rel weight in ♂ (23%) Spleen: (↓) abs weight in ♀ (9%) and (↑) rel weight in and ♀ (4%) Adrenals: (↓) abs weight in ♀ (9%) Testes: (↑) rel weight in ♂ (7%) <u>Macroscopic pathology:</u> Kidney: ▪ Pale in colour in ♂ (12/12) ▪ Enlargement in ♂ (12/12) <u>Histopathology:</u> Kidney: ▪ Focal tubular atrophy in ♂ (12/12) ▪ Dilation of proximal tubules in ♂ (12/12) 1000 ppm (57.1 ♂/61.5 ♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> (↓) bw in ♂ [week 3 (2%), week 8 (4%),week 9 (4%), week 10 (4%), week 11 (4%) and week 12 (4%)] (↓) food consumption in in ♀ [week 1 (12%)] <u>Clinical chemistry:</u> (↑) T chol in ♀ (11% ncdr) (↓) GPT in ♀ (14% ncdr) (↑) P in ♀ (13% ncdr) <u>Organ weights:</u> Liver: (↑) rel weight in ♂ (5%) 200 ppm (11.7 ♂/12.8 ♀ mg/kg bw/day) <u>Clinical chemistry:</u> (↑) T chol in ♀ (10% ncdr) 40 ppm (2.3 ♂/2.5 ♀ mg/kg bw/day) <u>Clinical chemistry:</u> (↑) T chol in ♀ (8% ncdr) (↑) P in ♀ (17% ncdr) **NOAEL** was determined to be **1000 ppm for males (57.1 mg/kg/day) and females (61.5 mg/kg/day).** Based on lower hematocrit and haemoglobin values as well as increased liver and kidney weights and decreased bodyweights and food consumption in both sexes, increased T chol in, GGTP and ALP in males, and kidney pathologies (*macro* and *histo*) in males; all at 5000ppm (LOAEL)	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
SL-160 technical: 13-Week oral subchronic toxicity study in dogs Method: OECD 409 (1998) GLP: Yes Deviations from current test guideline (OECD TG 409, 1998): uterus, epididymis and gall bladder weights were not recorded. Dog strain: Beagle dog 4 dogs/sex/dose **Study acceptable** STOT RE 1 ≤ 10 mg/kg bw/day STOT RE 2 ≤ 100 mg/kg bw/day	Purity: 97.3% Oral (gelatin capsules). Doses: 2, 10, 50, 250 mg/kg/day (m) and 2, 10, 50, 100 mg/kg/day (f). Daily for 13 weeks. Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, biochemistry, organ weights, urianalysis, ophthalmology, gross pathology and histopathology.	Mortality: In the 250 mg/kg/day group, one male showed no stool and decreased spontaneous motor activity, and was killed in extremis at week 11. 250 mg/kg/day (m) Clinical signs: Vomit of feed at Weeks 1 and 2 of treatment (Animal No. 19) and Week 5 of treatment (Animal No. 18), mucous stool at Week 5 of treatment Animal No. 17) and Week 10 of treatment (Animal No. 19) , swelling of the foreleg at Week 10~11 of treatment (Animal No. 19), an d a mass of the auricle from Week 5 of treatment to the end of the study (Animal No. 19). Clinical chemistry: (↑) ALP in ♂ [week 7 (112%)] (↑) GOT in ♂ [week 7 (5433%)] (↑) GPT in ♂ [week 7 (3142%)] (↓) creatin ♂ [week 7 (36%)] (↓) BUN ♂ [week 7 (21%)] (↓) albumin in ♂ [week 7 (18%) and week 13 (18%)] (↑) TG in ♂ [week 4 (85%), week 7 (150%) and week 13 (109%) (↓) Ca in ♂ (6%) Organ weights: Liver: (↑) abs wt in ♂ (73%) and (↑) rel wt in ♂/(81%) Kidney: (↑) abs wt in ♂ (32%) Thyroid: rel wt in ♂ (52%) Macroscopic pathology: Liver: ▪ Enlargement in ♂ (3/3 terminal kill) Skeletal muscle: ▪ Pale in colour in ♂ (3/3 terminal kill) Histopathology: Liver ▪ Difuse hepatocellular swelling in ♂ (3/3, terminal kill) ▪ Increased deposition of brown pigment in ♂ (3/3, terminal kill) Spleen: ▪ Increased deposition of brown pigment in ♂ (3/3, terminal kill) Thymus: ▪ Atrophy in ♂ (3/3, terminal kill) Skeletal muscle: ▪ Atrophy/degeneration in ♂ (3/3, terminal kill) 100 mg/kg/day (f) Clinical signs: one female (Animal No. 117) showed diarrhea/loose stool at Week 10 of treatment and a subcutaneous mass of the abdominal region at Week 13 of treatment. Haematology: (↓) Activated partial thromboplastin time (APTT) in ♀ at weeks 4 (8%), 7 (10%) and 13 (9%). Clinical chemistry: (↓) albumin in ♀ (8%) (↑) P in ♀ [week 7 (15%) and week 13 (14%)]	Anonymous 42 (1994) (CA) B.6.3.2.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
		Histopathology: Liver ▪ Difuse hepatocellular swelling in ♂ (4/4) 50 mg/kg/day Clinical signs: one male each showed vomit of feed at Week 2 of treatment (Animal No. 13) and Week 9 of treatment (Animal No. 16). One female (Animal No. 113) exhibited diarrhea/loose stool at Week 9 of treatment. Histopathology: Liver ▪ Increased deposition of brown pigment in ♂ (4/4) 10 mg/kg/day Clinical signs: one female (Animal No. 112) showed diarrhea/loose stool at Week 9 of treatment and swelling of the foreleg at Weeks 7-8 of treatment. Histopathology: Liver ▪ Increased deposition of brown pigment in ♂ (4/4) 2 mg/kg/day Clinical signs: one male (Animal No. 7) showed vomit of feed at Week 1 of treatment. **NOAEL was 2 mg/kg bw/day for males** based on liver histopathology at 10 mg/kg bw.day **and 50 mg/kg bw/day for females** based on haematology and clinical chemistry at 100mg/kg bw/day	
A range-finding study for a 21-day repeated dose dermal toxicity study in Albino rabbits vith technical SL-160 Method: US EPA FIFRA Guideline No 82-2. GLP: Yes Deviations from current test guideline: study lasted for 21 days instead of 28 Rabbit strain: New Zealand White rabbit 2 rabbits/sex/dose Statistics not performed **Preliminary test** Guideline value for	Purity: 97.1% Dermal application of the test material moistened with tap water. Doses: 125 , 250, 500, 750, or 1000 mg/kg/day Daily for 21 days, 6 hours per day. Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, biochemistry, organ weights, gross pathology and histopathology.	Mortality: No mortality was observed. No other signs of toxicity were noted during the 21-day study period. In addition, no effects on bodyweight, bodyweight gain or food consumption were observed in any of the test groups when compared with the controls. Clinical pathology parameters and organ weights were comparable among control and test groups, and no treatment-related gross or microscopic changes were noted in the tissues of test animals when compared with the controls. **NOAEL 1000 mg/kg/day**	Anonymous 43 (1994) (CA) B.6.3.3.1

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
classification extrapolated to 3-week study (dermal): STOT RE 1 ≤ 85.7 mg/kg bw/day STOT RE 2 ≤ 857.1 mg/kg bw/day			
A 21-day repeated dose dermal toxicity study in albino rabbits with technical SL-160 Method: 92/69/EEC part B9 GLP: Yes Deviations from current test guideline: study lasted for 21 days instead of 28 Rabbit strain: New Zealand White rabbit 5 rabbits/sex/dose **Study acceptable** Guideline value for classification extrapolated to 3-week study: STOT RE 1 ≤ 85.7 mg/kg bw/day STOT RE 2 ≤ 857.1 mg/kg bw/day	Purity: 96.3% Dermal application of the test material moistened with tap water. Doses: 250, 500, or 1000 mg/kg/day Daily for 21 days, 6 hours per day. Parameters observed: mortality, clinical signs, bodyweight and food intake, haematology, biochemistry, organ weights, gross pathology and histopathology.	Mortality: No mortality was observed. 500 mg/kg/day (↑) abs and rel food consumption in ♀ [week 2 (27%)] and [week 0 (20%) and week 2 (21%)] **NOAEL 1000 mg/kg/day**	Anonymous 44 (1994) (CA) B.6.3.3.2
A 90-Day Dietary Neurotoxicity Study of Flazasulfuron in Rats Method: OPPTS 870.6200 GLP: Yes Deviations from current test guideline: None	Purity: 96.3% Oral (diet) Doses of 0, 300, 3000, and 10000 ppm equivalent to 0, 19/22, 190/229, and 649/732 mg/kg bw/day for ♂/♀ Daily for 13 weeks Parameters observed:	Mortality: No mortality was observed. 10000 ppm (649 ♂/732 ♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> (↓) abs bw in ♂ [week 1 (18%), week 2 (18%), week 3 (18%), week 4 (20%), week 5 (20%), week 6 (22%), week 7 (21%), week 8 (22%), week 9 (21%), week 10 (21%), week 11 (22%), week 12 (21%), week 13 (21%)] and in ♀ [week 7 (8%), week 8 (9%), week 9 (9%), week 10 (9%), week 11 (9%), week 12 (9% ns), week 13 (8%)] (↓) terminal bw in ♂ (19%) (↓) abs bw gain in ♂ [week 1 (76%), week 2 (19%), week 3 (19%),	Anonymous 45 (2012) (CA) B.6.7.1.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
Rat strain: Crl:CD(SD) rats 12 rats/sex/dose **Study acceptable** STOT RE 1 ≤ 10 mg/kg bw/day STOT RE 2 ≤ 100 mg/kg bw/day	mortality, clinical signs, bodyweight and food intake, , brain weight, gross pathology, neuropathology and neurobehavioural assessment.	week 4 (30%), week 5 (30%), week 6 (43%), week 11 (47%), week 13 (86%)] and in ♀ [week 1 (53%)] (↓) total bw gain in ♂/♀ (32%/21%) (↓) food consumption in ♂ [week 1 (29%), week 2 (15%), week 3 (18%), week 4 (18%), week 5 (15%), week 6 (18%), week 7 (21%), week 8 (19%), week 9 (18%), week 10 (18%), week 11 (15%), week 12 (18%), week 13 (15%)] and in ♀ [week 3 (11%), week 4 (11%), week 5 (11%), week 6 (6%), week 7 (11%), week 8 (12%), week 10 (17%), week 11 (17%), week 13 (18%)] (↓) total food consumption in ♂/♀ (18%/12%) (↓) rel food consumption in ♂ [week 1 (20%), and (↑) week 12 (6%) and week 13 (6%). (↓) in ♀ [week 4 (8%) and week 13 (8% ncdr)] Motor activity: (↑) ambulatory counts in ♀ [week 7, mins 31-40 (127%) and mins 51-60 (251%)] Organ weights: (↓) abs brain wt in ♀ (5%) considered not test substance-related 3000 ppm (190 ♂/229 ♀ mg/kg bw/day) Motor activity: (↑) ambulatory counts in ♀ [week 7, mins 51-60 (188%)] Organ weights: (↓) abs brain wt in ♀ (4%) considered not test substance-related NOAEL 10000 ppm (649/743 mg/kg bw/day)	
2-week feeding study in mice Non-guideline study GLP: No Mice strain: SPF Fischer rats (F344/DuCrj) 8 males/dose Only 6 animals per group were subjected to histopathology examination **Study acceptable** The method was designed to provide additional information for a Guideline required study STOT RE 1 ≤ 65	Test substance: **Flazasulfuron [**SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.3% purity **Positive control: d-limonene** Oral (diet) **Doses:** **Flazasulfuron** Males: 0, 400, 800 mg/kg bw/day. **d-limonene** (positive control) Males: 1500 mg/kg bw/day	Mortality*:* 2 males from high dose group died on 7 and 8 days of treatment. Clinical signs*:* The two animals died in the high dose group showed decreased spontaneous motor activity, bradypnea, pale colored eye, and pale colored skin. In addition, loose stool was found in 3 animals and statistically significant increase incidences of soiled fur was observed in all animals (8/8 animals vs 0/8 in controls). In the positive control group (d-limonene), a statistically significant increase incidences of loose stool (5/8 vs 0/0 in controls) and soiled fur (8/8 animals vs 0/8 in controls) were observed compared with controls. There were no deaths during the treatment period. **800 mg/kg bw/day** Bodyweight: ▪ (↓) bw in at week 1 (14%) and 2 (8%). Organ weight Liver: (↑) abs and rel wt (32% and 42%). Kidney: (↑) abs (17%, n.s.) and rel wt (26%). Histopathology ▪ (↑) Hyaline droplets deposition in kidney proximal tubular cells (2/6 animals vs 0/6 in controls). **400 mg/kg bw/day** Organ weight	Anonymous 46 (1995) (CA) B.6.8.2.3

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
mg/kg bw/day STOT RE 2 ≤ 650 mg/kg bw/day	2-weeks feed exposure.	Liver: (↑) abs and rel wt (23% and 24%). Kidney: (↑) abs and rel wt (16% and 17%). Histopathology ▪ (↑) Hyaline droplets deposition in kidney proximal tubular cells (6/6 animals vs 0/6 in controls). **d-limonene positive control group (1500 mg/kg bw/day)** Bodyweight: ▪ (↓) bw in at week 1 (12%) and 2 (10%). Organ weight Liver: (↑) abs and rel wt (27% and 40%). Kidney: (↑) abs and rel wt (18% and 33%). Histopathology ▪ (↑) hyaline droplets deposition in kidney proximal tubular cells (6/6 animals vs 0/6 in controls). ▪ (↑) Centrilobular hepatocellular swelling (6/6 animals vs 0/6 in controls). **NOAEL** not derived due to adverse effects were observed in the low dose level.	
Chronic toxicity study in dogs **(1-year)** Method: US EPA 83-1 and OECD TG 452 (1981) GLP: Yes Deviations from current test guideline (OECD TG 452, 2018): -No satellite groups to monitor the reversibility of toxicological changes were incorporated. -Food efficiency was not measured. -Organ weight of epididymides and uterus were not measured. -No historical control data provided. Beagle dogs Males and females 4/sex/dose group	Test substance: **Flazasulfuron [**SL-160 technical or 1-(4,6-dimethoxyprymidin-2-yl)-3-[(3-trifluoromethylpyridin-2-yl)sulphonyl]urea]; 97.3% purity Oral (gelatine capsules) Doses: Males: 0, 0.4, 2, 10 and 50 mg/kg bw/day. Females: 0, 2, 10 and 50 mg/kg bw/day. 52-weeks. Daily oral administration.	Mortality**:** There were no deaths in any groups of either sex during the treatment period. Clinical signs**:** Vomit of feed, and vomit of foamy fluid were detected in all animals of both sexes (included control group); and bloody discharge from vulva was commonly occurred in all female dog. **50 mg/kg bw/day** ♂/♀ Bodyweight: *Bodyweight gain not measured.* ▪ (↓) bw in ♂ (3-5%; n.s.) and ♀ (3-4%; n.s.) throughout week 16-termination. Clinical biochemistry: ▪ (↑) alkaline phosphatase (ALP) in ♂ at week 26 (103%; n.s.) and week 52 (52%; n.s.). ▪ (↑) glutamic pyruvic transaminase (GPT) in ♂ at week 26 (148%; n.s.) and week 52 (93%; n.s.). ▪ (↓) inorganic phosphorus (P) in ♀ before initiation treatment (11%, ndr). Urinalysis ▪ (↓) urine volume in ♀ at week 13 (49%, ncdr). ▪ (↓) urine volume in ♂ at week 26 (40%, n.s.; ncdr) and 52 (54%, n.s.; ncdr). Organ weight: ▪ Thyroid: (↓) abs (27%, ndr) and rel (22%; n.s.; ndr) wt in ♂ within HCD. **Male absolute and relative thyroid weights**	Anonymous 22 (1995b) (CA) B.6.5.3

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, route of exposure, dose levels, duration of exposure</th><th>Results

[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>Study acceptable

STOT RE 1 ≤ 2.5 mg/kg bw/day

STOT RE 2 ≤ 25 mg/kg bw/day</td>
<td></td>
<td>
<table>
<tr><th>Dose (mg/kg bw/day)</th><th>Number of animals examined</th><th>Absolute thyroid wt (mean+S.D.)</th><th>Relative thyroid wt.
(mean+S.D.)</th></tr>
<tr><td>Historical control</td><td>30</td><td>840±212</td><td>0.0071±0.0017</td></tr>
<tr><td>0</td><td>4</td><td>1152±152*</td><td>0.0101±0.0013**</td></tr>
<tr><td>0.4</td><td>4</td><td>997±237</td><td>0.0087±0.0021</td></tr>
<tr><td>2</td><td>4</td><td>796±164</td><td>0.0068±0.0008</td></tr>
<tr><td>10</td><td>4</td><td>840±106</td><td>0.0072±0.0014</td></tr>
<tr><td>50</td><td>4</td><td>842±80</td><td>0.0079±0.0008</td></tr>
</table>
* statistically significantly different from the historical control p = <0.05
** statistically significantly different from the historical control p = < 0.01

<u>Necropsy:</u>
Liver:
▪ (↑) incidence nodules, scar and incisures in ♂ (25%; 1/4 animals vs 0/4 in controls; n.s.).
▪ (↑) incidence enlargement in ♀ (25%; 1/4 animals vs 0/4 in controls; n.s.).

<u>Histopathology:</u>
Liver:
▪ (↑) inflammatory cell infiltration in centrilobular/periportal in ♂ (50%; 2/4 animals vs 0/4 in controls; n.s.), and in ♀ (75%; 3/4 animals vs 0/4 in controls; n.s.).
▪ (↑) bile ductal proliferation in ♂ (25%; 1/4 animals vs 0/4 in controls; n.s.).
▪ (↑) hepatocellular necrosis ♂ (25%; 1/4 animals vs 0/4 in controls; n.s.).
▪ (↑) centrilobular hepatocellular swelling in ♀ (25%; 1/4 animals vs 0/4 in controls; n.s.).

10 mg/kg bw/day ♂/♀
<u>Haematology:</u>
▪ (↑) platelet in ♀ before initiation treatment (28%, ndr).
▪ (↑) WBC in ♀ at week 52 (40%, ndr).

<u>Urinalysis</u>
▪ (↓) urine volume in ♀ at week 13 (21%; n.s.; ncdr).

<u>Histopathology:</u>
Liver:
▪ (↑) inflammatory cell infiltration in centrilobular/periportal area in ♂ (100%, 4/4 animals vs 0/4 in controls) and in ♀ (75%; 3/4 animals vs 0/4 in controls; n.s.).
▪ (↑) bile ductal proliferation in ♂ (25%; 1/4 animals vs 0/4 in controls; n.s.).
▪ (↑) lymphoid hyperplasia around bile duct in ♀ (25%; 1/4 animals vs 0/4 in controls; n.s.).

2 mg/kg bw/day ♂/♀
<u>Clinical biochemistry:</u>
▪ (↑) total bilirubin (T. Bil.) in ♂ at week 26 (18%, ncdr).

<u>Urinalysis</u>
▪ (↓) urine volume in ♀ at week 13 (52%, ncdr).
</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		Histopathology: Liver: ▪ (↑) inflammatory cell infiltration in centrilobular/periportal area in ♂ (25%; 1/4 animals vs 0/4 in controls; n.s.). ▪ (↑) lymphoid hyperplasia around bile duct in ♀ (25%; 1/4 animals vs 0/4 in controls; n.s.). **0.4 mg/kg bw/day ♂** Haematology: ▪ (↑) monocytes in ♂ at week 26 (300%, ndr). Clinical biochemistry: ▪ (↑) total bilirubin (T. Bil.) in ♂ at week 52 (18%, ncdr). **$NOAEL_{toxicity}$: 2 mg/kg bw/day for ♂ and ♀.**	

Table 106: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 107: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
See summary in this section				

Other studies relevant for STOT RE

Other long-term exposure studies, such as on carcinogenicity, or reproductive toxicity studies, can also provide evidence of specific target organ toxicity that could be used in the assessment of classification.

Chapter 10.9: 2-year long term toxicity and carcinogecity study in rats and an 18-month oncogenicity study in mice.

Chapter 10.10: multigeneration study in rats, teratology study in rats and teratology study in rabbits.

These studies are properly summarised in the corresponding chapters. Effects observed in carcinogenicity and reproductive studies are included in the following *section 10.12.1*.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Studies in rats:

In a 2-week dose study in rats (Anonymous 46, 1995; B.6.8.2.3), alterations were observed in kidney in the two dose groups tested with flazasulfuron (400 and 800 mg/kg bw/day dose groups). Increase in absolute and relative weights were recorded in kidney and liver in both treated groups, however, histopathological

examinations only revealed findings in the kidney. Increased incidences of hyaline droplet deposition in proximal tubular cells were noted in both treated groups (6/6 animals and 2/6 animals examined in 400 and 800 mg/kg bw/day groups, respectively), compared with control group (0/6 animals examined). Moreover, the hyaline droplets observed in the proximal tubular cells were positive for α2µ-globulin immunohistochemistry.

The extrapolated cut-off value for STOT RE 2 classification for a 2-week dose-repeated study is 640 mg/kg bw/day. However, despite of effects observed in the study are below the cut-off values for STOT RE 2, and since α2µ-globulin is a male rat-specific protein, and hyaline droplet accumulation or the spectrum of lesions comprising α2µ-globulin nephropathy has not been observed in female rats or mice of either sex, it must therefore be concluded that this mechanism is not relevant for human hazard assessment and consequently, classification for STOR RE is not required.

In a 4-week dose range finding study in rats (Anonymous 39, 1988a; B.6.3.1.2) the main target organ was the liver, which showed alterations in the absolute and relative weights in males from the dose of 1000 ppm (72.2 (m)/88.0 (f) mg/kg bw/day) and in females from the dose of 5000 ppm (424 mg/kg bw/day; 354 mg/kg bw/day for males). However, at the lower dose, these variations were less than 10% and in males there was not a clear dose relationship. In addition, no histological findings were observed in liver (centrilobular hepatocyte swelling in males) up to the dose of 10000 ppm (731/834 mg/kg bw/day). Therefore, the toxicological significance of these variations is doubtful. At the highest dose, [20000 ppm (1145 /1544 mg/kg bw/day)] histological alterations included enlargement of the liver in both sexes. Decreased values in hematocrit, hemoglobin and/or erythrocyte counts at 5000 ppm and higher were considered to be treatment related and indicative of anemia. Statistically significantly altered spleen mean weight, and heart and testes relative weight were also observed at terminal necropsy in males from 5000 ppm; and in brain relative weight (not clearly dose related).

The extrapolated cut-off value for STOT RE 2 classification for a 4-week dose-repeated study is 321.4 mg/kg bw/day. Consequently the mentioned effects in liver from the dose of 10000 ppm (731/834 mg/kg bw/day) are not regarded for STOT RE classification.

In a 13-week oral subchronic toxicity study in rats (Anonymous 41, 1988b; B.6.3.2.1) alterations in relative liver weight were observed in males from the dose of 1000 ppm (57.1/61.5 mg/kg bw/day). No histological adverse effects were seen at this dose. At the maximum dose of 5000 ppm (287/309 mg/kg bw/day) absolute and liver weight were increased in both sexes. No alterations were observed in liver histology. Kidney absolute and relative weights were increased at 5000 ppm. In addition, all males showed paleness in colour, enlargement, focal tubular atrophy and dilation of proximal tubules in this organ.

It has to be noted that 287/309 mg/kg bw/day is clearly above the cut-off value for STOT RE 2 classifications for a 90-day dose-repeated study (100 mg/kg bw/day). Consequently effects are not regarded for STOT RE classification.

In a long-term toxicity and carcinogenicity study in rats (Anonymous 20, 1995a; B.6.5.1) changes in kidneys, testes, parathyroid, eye and aorta were observed in males at 400 ppm (13.26 (m)/16.45 (f) mg/kg bw/day) and higher and in females at 4000 ppm (172.6 mg/kg bw/day; dose only given to females). Males at 2000 ppm also exhibited changes in the liver, stomach, aorta, parathyroid and eyes.

For males at 2000 ppm group, a statistically significant increase in incidences of emaciation, decreased motor activity, bradypnea, pale and opaque eyes, and pale-coloured skin was observed. For females at 4000 ppm group, a statistically significant increase in hair loss and soiled fur on external genital area was observed, but only soiled fur was associated to treatment, because of an increase of urine volume was observed in this group. No treatment-related differences in incidence of clinical signs between the control and the treated groups were detected for both sexes at 400 ppm or lower doses.

The primary effects were noted in the kidneys in males from 400 ppm on (including early changes of chronic nephropathy, increased brown pigment deposition of proximal tubular cells, increased hyaline droplets of proximal tubular cells, increased hyaline droplets of proximal tubular cells, luminal dilation of proximal tubule and pelvic epithelial hyperplasia). In females, at 4000 ppm, early change of chronic nephropathy, increased brown pigment deposition of proximal tubular cells, and luminal dilation of proximal tubules were also

observed. The incidence of effects in the kidney were not only increased with increasing dose, but also the incidence increased over time.

Effects in males are considered to be related to chemically-induced hyaline droplet nephropathy of proximal tubular cells involving α2u-globulin at the doses of 400 and 2000 ppm. However, at the dose of 2000 ppm (70.1 mg/kg bw/d), at or before week 13 the cut-off value is 100 mg/kg/day. At this week, there are no histopathological effects in kidney. At the dose of 400 ppm (13.26 mg/kg bw/d), effects are relevant if they are seen at or before week 52 (cut-off value of 25 mg/kg/day). At week 26 increased hyaline droplets of proximal tubular cells and early chronic nephropathy were seen in males at the dose of 13.26 mg/kg/day (cut off value of 50 mg/kg/day). However, these changes were not accompanied by alterations in the absolute or relative kidney weight, in the clinical chemistry or in urianalysis. At week 52, males showed increased hyaline droplets of proximal tubular cells, luminal dilation of proximal tubule and early chronic nephropathy, as well as an increase in absolute and relative kidney weights were observed at the dose of 13.26 mg/kg/day (cut off value of 25 mg/kg/day). In addition, there is an alteration in ALP levels.

The mentioned observed effects in males at weeks 26 and 52 are inside the range of STOT RE 2. Nevertheless, increased hyaline droplets of proximal tubular cells is not considered relevant for classification. In addition, luminal dilation of proximal tubule and early chronic nephropathy are considered concurrent to the first effect, since they are not seen in females. The reasons to consider increased hyaline droplets of proximal tubular cells not relevant are the following:

In the RAC opinion of (*RS*)-1-{1-ethyl-4-[4-mesyl-3-(2methoxyethoxy)-o-toluoyl]pyrazol-5-yloxy}ethyl methyl carbonate; tolpyralate, increased deposition of hyaline droplets in proximal tubular cells was observed in male rats below the general values for classification. Association of hyaline droplets with α2u-globulin has been demonstrated and alpha2u-globulin nephropathy is a mechanism specific to male rats. Therefore, RAC does not consider the increased incidence of hyaline droplet deposition in male rats to support classification.

In an EFSA opinion not related to pesticides (Scientific opinion on the evaluation of substances as acceptable previous cargoes for edible fats and oils), it is stated that 'an increased incidence of hyaline droplets was observed in kidneys of males at the two highest doses. These were not observed in female rats at the same dose levels. α2u-Globulin immunoreactivity was present in hyaline droplets of the renal proximal tubule epithelium of male rat kidneys. This mechanism is not relevant for human risk assessment.'

Therefore, despite histopathological effects have been seen in males below the cut-off values for STOT RE 2, available information points out that the mechanism is not relevant for classification.

In a range-finding study in rats for a two-generation reproductive study (Anonymous 23, 1993; B.6.6.1.1) no relevant effects for STOT RE classification were associated with treatment.

In the two-generation reproductive study in rats (Anonymous 24, 1995b; B.6.6.1.2) at the doses of 141 ♂/160 ♀ mg/kg bw/day and 717 ♂/801 ♀ mg/kg bw/day, parental and F1 animals showed histological alterations in kidney. However, these effects are seen at a dose clearly above the cut-off values for STOT RE 2. Therefore, no classification is regarded for STOT RE.

In a developmental toxicity study in Sprague Dawley rats (Anonymous 27, 1996; B.6.6.2.3) an increase in liver weight (8%) was found from the dose of 300 mg/kg/day in dams. Therefore, no classification is regarded for STOT RE.

In a 90-Day Dietary Neurotoxicity Study of Flazasulfuron in rats (Anonymous 45, 2012; B.6.7.1.2) a decrease in the absolute brain weight in females (considered not to be treatment related and at doses above 100 mg/kg/day) was observed. There were no relevant neurotoxic effects noted at any dietary exposure level.

Studies in mice:

In a 6 week feeding study in mice (Anonymous 38, 1992; B.6.3.1.1) adverse effects in liver were observed at the two highest tested levels of 5000 ppm (equivalent to 884 and 1032 mg/kg bw/day in males and females, respectively) and 10000 ppm (equivalent to 1745 and 2036 mg/kg bw/day in males and females, respectively). Centrilobular hepatocyte hypertrophy was observed in both sexes at both doses. In addition, at the dose of 5000 ppm, statistically significant increases from 12% to 35% in the absolute liver weight in males and in the relative weight in both sexes were seen; and at the highest dose, increases of the absolute and relative liver

weight in both sexes. In addition, a no-statistically significant increase in cholesterol levels in males was observed from the dose of 5000 ppm, as well as an increase of 10% in the albumin in this sex at the top dose. The (A/G) ratio was also increased in males and females at this dose.

The dose of 5000 ppm (equivalent to 884 and 1032 mg/kg bw/day in males and females, respectively) is clearly above the extrapolated cut-off value for a 6-week study for STOT RE 2 (214.3 mg/kg bw/day). Consequently, effects at this dose cannot be regarded for STOT RE classification.

In an oncogenicity study in mice (Anonymous 21, 1995a; B.6.5.2) increased incidence of hepatocellular hypertrophy was observed in both sexes at the dose of 7000 ppm (987.4 ♂/1165.5 ♀ mg/kg bw/day), as well as an increased incidence of severity and pigment in males. An increase in liver relative weight in both sexes was also observed. At the dose of 3500 ppm (497.8 and 596.4 mg/kg bw/day in males and females, respectively), hepatocellular hypertrophy accompanied by an increase in absolute and relative liver weight was observed. However, these effects are seen at a dose clearly above the cut-off values for STOT RE 2. Therefore, no classification is regarded for STOT RE.

Studies in dogs:

In a 4-week oral toxicity study in dogs (Anonymous 40, 1992; B.6.3.1.3) alterations were observed from the dose of 10 mg/kg/day in liver relative weight in males; and in females, in heart and brain relative weight; and in pancreas and adrenals both absolute and relative weight. Adrenal cortical hyperplasia was found in the studied females from the dose of 100 mg/kg/day. Histological alterations in liver were found from the dose of 500 mg/kg/day.

The extrapolated cut-off value for STOT RE 2 classification for a 4-week dose-repeated study is 321.4 mg/kg bw/day. Consequently, the mentioned effects in the adrenal from the dose of 100 mg/kg bw/day may be considered for STOT RE classification.

In a 13-week oral subchronic toxicity study in dogs (Anonymous 42, 1994; B.6.3.2.2) brown deposition pigment was seen in the liver of all males from the dose of 10 mg/kg/day. However, this alteration did not show a clear relationship with significant variations of absolute and relative liver weights. At the dose of 250 mg/kg/day (only given to males) increases in the absolute and relative liver weight were accompanied by enlargement. This effect was not dose-dependent in females (3/4 and 2/4 at the top doses of 50 and 100 mg/kg/day). It is considered that this brown deposition was derived from erythrocyte breakdown, consistent with the decreases seen in haematocrit levels, and haemoglobin and erythrocyte concentrations (anaemia). However, there is no indication that this pigment deposits can impair liver function or it is associated with severe hepatic damage. In males, effects in biochemistry were seen at the highest dose of 250 mg/kg/day (ALP, GOT, GPT, creatine, BUN, A/G ratio, albumin, tryglicerides, calcium, chloride). At the dose of 50 mg/kg/day, only an isolated reduction of albumin was seen. In females, at the highest dose of 100 mg/kg/day, a decrease in albumin and an increase in inorganic phosphorous were observed.

Diffuse hepatocellular swelling were observed both in males and females at the highest dose. This may indicate the beginning of hepatic damage. Hepatocellular degeneration/necrosis was not dose-dependent in any sex.

A clear pattern of hepatotoxicity which shows severe damage in the range of doses for STOT RE according to the histological findings was not observed. In addition, there is no correlation with other observations as clinical chemistry or alterations in the organ weight.

On the other hand, an atrophy in thymus was observed in males from the dose of 50 mg/kg/day (cut-off value of 100 mg/kg/day). However, this effect was not seen at the dose of 50 mg/kg/day in the one year study in dogs.

In a 12-month oral chronic toxicity study conducted in beagle dogs (Anonymous 22, 1995b; B.6.5.3), flazasulfuron was tested at dose levels of 0, 0.4, 2, 10 and 50 mg/kg bw/day for males, and 0, 2, 10 and 50 mg/kg bw/day for females.

The rationale for selection of dose levels in this study arises from a previous 13-week oral subchronic toxicity study (Anonymous 42, 1994; B.6.3.2.2), conducted in 4 beagle dogs per group at dosage levels of 0, 2, 10, 50, and 250 mg/kg bw/day for males, and 0, 2, 10, 50, and 100 mg/kg bw/day for females. In this previous study, several grades of anemic and hepatotoxic changes were observed at 250 and 100 mg/kg bw/day groups for

males and females, respectively. In addition, the histopathological examinations revealed atrophy/degeneration of the thymus and/or the skeletal muscle. One male in the 250 mg/kg bw/day group had marked decrease in bodyweight and food consumption, decreased spontaneous motor activity and no stool, and was killed in extremis at week 11 of treatment. The 50 mg/kg bw/day male and female groups replicated the hepatotoxic alterations detected in the clinical biochemistry and histopathology analysis of high dose groups. Furthermore, atrophy of the thymus was noted in three males and one female at 50 mg/kg bw/day. In the 10 mg/kg bw/day group, an increasing trend in glutamic pyruvic transaminase (GPT) value, together with hepatotoxic alterations displayed in the histopathologic analysis were detected. Taken into account these results, the maximum dosage of 50 mg/kg bw/day dose was established for the 12 months chronic study.

In the present study, no mortality or morbidity signs were observed at any dose level for both sexes. All the animals survived until study termination with good general conditions.

A slight decreased bodyweight was observed at 50 mg/kg bw/day for males (3-5%) and females (3-4%) from week 16 until the end of study. This tendency was no significant, but relevant due to was associated to treatment. Food consumption was not altered in any tested animals, except in one female in the 50.0 mg/kg bw/day group at week 16. The poor appetite was likely attributable to the liver dysfunction as indicated by higher levels of alkaline phosphatase (ALP) and glutamic pyruvic transaminase (GPT) in the clinical analysis at week 17. However, the appetite of the animal improved gradually and reached normal values 4 weeks later.

No ophthalmologic dysfunctions were observed in any group dosage.

Statistically significant decrease in urine volume was detected at weeks 13 in females at 50 mg/kg bw/day (49%) and 2 mg/kg bw/day (52%) groups, and a slight decrease in the 10 mg/kg bw/day (21%) dose group, compared with control group. Besides, males at 50 mg/kg bw/day presented a non-statistically significantly low levels of urine volume at week 26 (40%) and 52 (54%). However, since these animals had neither abnormal changes in other parameters in urinalysis nor specific pathological findings in the urinary tract, it is unlikely that these changes were treatment-related.

Haematological examinations showed few statistically significant deviations. All of them were considered to be incidental, because they were neither dosage-related nor time-related.

Clinical biochemistry results revealed an increasing trend in alkaline phosphatase (ALP) and glutamic pyruvic transaminase (GPT) parameters in 50 mg/kg bw/day male group at week 26 (103% and 148% for ALP and GPT, respectively) and 52 (52% and 93% for ALP and GPT, respectively) above cut-off values, which were consequence of hepatotoxicity of test substance and correlated with subsequent histopathologic liver observations. Similar results were observed in the higher dose (50.0 mg/kg bw/day or more) groups of both sexes in the 13-week oral subchronic toxicity study (Anonymous 42, 1994; B.6.3.2.2).

Statistically significant decrease in absolute thyroid weight were observed at 50 (27%), 10 (27%) and 2 (31%) mg/kg bw/day male groups, and a slight decrease at 0.4 mg/kg bw/day group, compared with control group. Lower relative thyroid weight was also statistically significant at 10 (29%) and 2 (33%) mg/kg bw/day male group, and a decreased trend was detected at 50 mg/kg bw/day male group. However, these results displayed in all treated groups were similar to historical control data of beagle dogs. Moreover, no histopathological alterations were detected in this organ and therefore, these differences were not attributable to test substance administration. In conclusion, MSCA deems the need for clarification of the increase of mean thyroid weight of control groups observed in the present study compared with historical control data.

On the other hand, the histopathological examinations revealed hepatotoxic changes such as inflammatory cell infiltration in 50% of male and 75% of female dogs at 50 mg/kg bw/day dose groups, in 100% of male and 75% of female dogs at 10 mg/kg bw/day dose groups, and in 25% of male dogs at 2 mg/kg bw/day; hepatocellular necrosis in 25% of high dose male dogs; hepatocellular swelling in 25% of high dose female dogs; bile ductal proliferation in 25% of males at 50 and 10 mg/kg bw/day dose group; and lymphoid hyperplasia around bile duct in 25% of female dogs at 10 and 2 mg/kg bw/day. These observations showed a dose-response pattern.

NOAEL for toxicity was considered **2 mg/kg bw/day for males and females**.

Studies in rabbits:

In a range-finding study for a 21-day repeated dose dermal toxicity study in Albino rabbits (Anonymous 43 1994; B.6.3.3.1) no effects were associated with treatment.

In a 21-day repeated dose dermal toxicity study in albino rabbits (Anonymous 44, 1994; B.6.3.3.2) no relevant effects for STOT RE classification were associated with treatment.

In a teratology study in rabbits (Anonymous 28, 1988c; B.6.6.2.4) no relevant effects for STOT RE classification were associated with treatment.

10.12.2 Comparison with the CLP criteria

Classification for repeated dose toxicity depends on the type of effects and the dose at which the effects are observed. The CLP criteria state that STOT RE is assigned on the basis of findings of 'significant' or 'severe' toxicity within relevant dose levels. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health.

Table 108: Summary table of relevant effects for STOT RE classification

Dose levels and duration of exposure	Effest relevant for STOT RE [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
4-week dose range finding study in rats Doses: 7.5, 72.2, 354, 731 and 1145 mg/kg/day (m) and 8.9, 88.0, 424, 834 and 1544 mg/kg/day (f) for 4 weeks	**5000 ppm (354 ♂/424 ♀ mg/kg bw/day*)** Organ weights: Liver: (↑) abs wt in ♂/♀ (27% ncdr/12%) and (↑) rel wt in ♂/♀ (36% ncdr/21%) Kidney: (↑) rel wt in ♂/♀ (25% ncdr/11% ncdr) Clinical chemistry: (↓) ALP in ♂ (31% ncdr) and ♀ (22% ncdr) (↓) TG in ♂ (28%) (↑) T chol in ♀ (21% ncdr) **Extrapolated (for toxicity studies of greater or lesser duration than 90 days) equivalent guideline value for classification in STOT RE 2 (≤ 300 mg/kg bw/day)*	Anonymous 39 (1988a) (CA) B.6.3.1.2
13-week oral subchronic toxicity study in rats Doses of 2.3, 11.7, 57.1, 287 mg/kg/day (m) and 2.5, 12.8, 61.5, 309 mg/kg/day (f).	**5000 ppm (287 ♂/309 ♀ mg/kg bw/day*)** Clinical signs: (↓) bw in ♂ and in ♀, (↓) terminal bodyweight in ♀ (13%) and (↓) food consumption in ♂ Haematology: (↓) Hematocrit in ♂ (4%) (↓) Hemoglobin in ♀ (3%) Organ weights: Liver: (↑) abs weight in ♂ (13%) and ♀ (20%) and rel weight in ♂ (19%) and ♀ (16%) Kidney: (↑) abs weight in ♂ (52%) and ♀ (20%) and (↑) rel weight in ♂ (60%) and ♀ (16%) Clinical chemistry: (↑) T chol in ♂ (15%) and ♀ (10% ncdr) (↓) ALP in ♂ (14%) (↑) GGTP in ♂ (22%) Macroscopic pathology: Kidney: Pale in colour in ♂ (12/12) and enlargement in ♂ (12/12) Histopathology: Kidney: Local tubular atrophy in ♂ (12/12 and dilation of proximal tubules in ♂ 12/12) **guideline value for classification in STOT RE 2 (≤ 100 mg/kg bw/day)*	Anonymous 41 (1988b) (CA) B.6.3.2.1

Dose levels and duration of exposure	**Effest relevant for STOT RE** [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	**Reference**
Long-term toxicity and carcinogenicity study in rats Males: 1.3, 13.3 and 70.1 mg/kg bw/day. Females: 1.6, 16,4 and 172.6 mg/kg bw/day.	400 ppm (13.26 mg/kg bw/d*) ▪ Increased hyaline droplets of proximal tubular cells y early chronic nephropathy in ♂ at week 26 (cut-off value of 50 mg/kg bw/d for category 2). ▪ Increased hyaline droplets of proximal tubular cells, luminal dilation of proximal tubule and early chronic nephropathy, as well as increased abs (16%) and rel (18) kidney weight at 13.26 mg/kg bw/d (cut-off value of 25 mg/kg bw/d for category 2) in ♂ at week 52. **MSCA opinion**: It is considered that increased hyaline droplets of proximal tubular cells is not relevant for classification since the mechanism is specific of male rats. **Extrapolated (for toxicity studies of greater or lesser duration than 90 days) equivalent guideline value for classification in STOT RE 2 (≤ 25 mg/kg bw/day regarding 1-year of duration for chronic toxicity)*	Anonymous 20 (1995a) (CA) B.6.5.1
13-week oral subchronic toxicity study in dogs Doses of 2, 10, 50, 250 mg/kg/day (m) and 2, 10, 50, 100 mg/kg/day (f).	**10 mg/kg/day for males*** Histopathology: Liver: Increased deposition of brown pigment in ♂ (4/4) **100 mg/kg/day for females**** Clinical chemistry: (↑) P in ♀ [week 7 (15%) and week 13 (14%)] Haematology: (↓) Activated partial thromboplastin time (APTT) in ♀ at weeks 4 (8%), 7 (10%) and 13 (9%) in females. Histopathology Difuse hepatocellular swelling in liver in ♂ (4/4). **MSCA opinion:** There is not a clear pattern of hepatotoxicity which shows severe damage in the range of doses for STOT RE according to the histological findings. In addition, there is no correlation with other observations as clinical chemistry or alterations in the organ weight. **guideline value for classification in STOT RE 2 (≤ 100 mg/kg bw/day)* ***guideline value for classification in STOT RE 1 (≤ 10 mg/kg bw/day)*	Anonymous 42 (1994) (CA) B.6.3.2.2
Oral chronic toxicity study in dogs (1-year) Doses of 0.4, 2, 10 and 50 mg/kg bw/day (m) and 2, 10 and 50 mg/kg bw/day (f).	**At 10 mg/kg bw/day*** Hematology: ▪ (↑) platelet in ♀ before iniciation treatment (28%, ndr), (↑) WBC in ♀ at week 52 (40%, ndr). Organ weights (end of treatment period): ▪ Thyroid: (↓) abs (27%) and rel (29%) wt in ♂ within HCD. Histopathology: ▪ Liver: (↑) inflammatory cell infiltration in centrilobular/periportal area in ♂ (100%, 4/4 animals) and in ♀ (75%; 3/4 animals; n.s.), (↑) bile ductal proliferation in ♂ (25%; 1/4 animals; n.s.), (↑) bile ductal proliferation in ♀ (25%; 1/4 animals; n.s.). **MSCA opinion:** Effects in liver do not show a clear dose-dependency and the severity is not enough to consider it for STOT RE classification. In addition, clinical chemistry effects do not show a relationship with histological alaterations. ** Extrapolated (for toxicity studies of greater or lesser duration than 90 days) equivalent guideline value for classification in STOT RE 2 (≤ 25 mg/kg bw/day and > 2.5 mg/kg bw/day)*	Anonymous 22 (1995b) (CA) B.6.5.3

Dose levels and duration of exposure	Effest relevant for STOT RE [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
2-week feeding study in mice **Doses**: 0, 400, 800 mg/kg bw/day (only males).	**400 mg/kg bw/day*** Organ weight Liver: (↑) abs and rel wt (23% and 24%). Kidney: (↑) abs and rel wt (16% and 17%). Histopathology ▪ (↑) Hyaline droplets deposition in kidney proximal tubular cells (6/6 animals vs 0/6 in controls). **Extrapolated (for toxicity studies of greater or lesser duration than 90 days) equivalent guideline value for classification in STOT RE 2 (≤ 640 mg/kg bw/day)*	Anonymous 46 (1995) (CA) B.6.8.2.3

10.12.3Conclusion on classification and labelling for STOT RE

The main target organs were liver and kidney. In the long-term toxicity and carcinogenicity study in rats, increased hyaline droplets of proximal tubular cells (at the dose of 13.26 mg/kg/day) were not considered relevant for classification because they were only seen in males, in which a specific mechanism of action is observed (and not relevant for humans). In addition, despite other effects were observed, as luminal dilation of proximal tubule and early chronic nephropathy, they are considered to be concurrent to the first effect. Therefore, despite this effect was seen below guidance value for STOT RE 2 (100 mg/kg bw/day), they are not considered for classification.

On the other hand, in the 13-week oral subchronic toxicity study in dogs, effects in liver histopathology below the cut-off values (50 mg/kg/day) are observed. Nevertheless, they are accompanied by clinical chemistry alterations only in males and a clear relationship with significant variations of absolute and relative liver weights was not seen. In addition, it is not considered that an impairment in liver function was produced. Therefore, these effects are not deemed relevant for STOT RE classification.

Taking into account the observed effects in kidney and liver, the MSCA considers that flazasulfuron does not cause adverse effects in these organs. The weight of the evidence, based on the whole available information on all studies in several species, indicate that flazasulfuron does not cause kidney or liver toxicity at dose levels below guidance values for STOT RE classification. Consequently, STOT RE classification is not proposed.

10.13 Aspiration hazard

Table 109: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

No evidence of aspiration hazard of flazasulfuron was found in the provided data.

10.13.2 Comparison with the CLP criteria

Although the definition of aspiration in *section 3.10.1.2* of Regulation (EC) No. 1272/2008 includes the entry of solids into the respiratory system, classification criteria for this hazard is established for liquid, aerosol and mist forms of a substance or a mixture.

Flazasulfuron is presented in a solid form, both pure (powder) and technical grade (granular solid), and therefore, no aspiration toxicity hazard is expected.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Data available indicates that flazasulfuron does not require classification for aspiration hazard.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Flazasulfuron is an herbicide active substance considered under Directive 91/414/EEC (subsequently Regulation 1107/2009) for representative use as a foliar spray. Available environmental fate and ecotoxicology studies have been considered and summarised in the original Draft Renewal Assessment Report, 2016 (RAR, Volume 3, Annex B8 and Annex B9) and in the renewal of approval dossier.

The key information pertinent to determine the environmental hazard classification for Flazasulfuron is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the representative test guideline, if applicable. Full robust summaries of these studies are presented in Annex 1 to this dossier.

11.1 Rapid degradability of organic substances

Table 110: Summary of relevant information on rapid degradability

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>Ready biodegradability.

OECD No. 301B</td><td>0% biodegradation in 28 days.

Results indicate Flazasulfuron is “not readily biodegradable”, at pH 7.4.</td><td>The study is considered acceptable</td><td>Anonymous (2014)</td></tr>
<tr><td>Hydrolysis
Flazasulfuron at pH 4, 5 and 7.

Guidelines: Not indicated.

FOCUS Degradation Kinetics (2006, 2011).</td><td>Flazasulfuron is not hydrolytically stable in solutions at pH 4, 5, 7 and 9 at 22ºC and 37ºC over a period of 30 days.

<u>Flazasulfuron:</u>
<table>
<tr><th></th><th>pH 5</th><th>pH7</th></tr>
<tr><td>DT₅₀ (days)</td><td>3.44-3.68</td><td>15.7-16</td></tr>
<tr><td>DT₉₀ (days)</td><td>11.4-12.2</td><td>52.3-53.2</td></tr>
</table>
<u>Metabolites:</u>

• DTPU
<table>
<tr><th></th><th>pH 5</th><th>pH7</th></tr>
<tr><td>DT₅₀ (days)</td><td>382-425</td><td>70.3-87.3</td></tr>
<tr><td>DT₉₀ (days)</td><td>>1000</td><td>233-290</td></tr>
</table>
• DTPP</td><td>The study is considered acceptable.

Kinetic evaluation of the raw data from Anonymous (1995).</td><td>Anonymous (1995)

Anonymous (2014).</td></tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td></td>
<td>
<table>
<tr><th></th><th>pH 5</th><th>pH7</th></tr>
<tr><td>DT₅₀ (days)</td><td>>1000</td><td>37.7</td></tr>
<tr><td>DT₉₀ (days)</td><td>>1000</td><td>125</td></tr>
</table>
TPSA
<table>
<tr><th></th><th>pH 5</th><th>pH7</th></tr>
<tr><td>DT₅₀ (days)</td><td>>1000</td><td>>1000</td></tr>
<tr><td>DT₉₀ (days)</td><td>>1000</td><td>>1000</td></tr>
</table>
ADMP
<table>
<tr><th></th><th>pH 5</th><th>pH7</th></tr>
<tr><td>DT₅₀ (days)</td><td>>1000</td><td>110</td></tr>
<tr><td>DT₉₀ (days)</td><td>>1000</td><td>364</td></tr>
</table>
</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic mineralisation in surface water.

OECD guideline 309.</td>
<td><u>Flazasulfuron</u>
DT₅₀: 21 and 25 days (at concentrations 0.002 mg/L and 0.010 mg/L respectively)
DT₉₀: 70 and 84 days (at concentrations 0.002 mg/L and 0.010 mg/L respectively)

Max. 0.6% mineralisation after 90 days.

<u>Metabolites:</u>
DTPU (SSRE-004)
DTPP (SSRE-005)</td>
<td>The study is considered acceptable.</td>
<td>Anonymous, (2014)</td>
</tr>
<tr>
<td>Aerobic aquatic metabolism in water/sediment systems.

BBA Guideline Part IV; EPA Guidelines 162-4; SETAC-Europe Guidelines (March 1995); Japan MAFF Guidelines.</td>
<td>DT₅₀ Whole system:
<table>
<tr><th></th><th>pH 6.6</th><th>pH 7.7</th></tr>
<tr><td>Parent</td><td>23.9d</td><td>23.9d</td></tr>
<tr><td>DTPU</td><td>-</td><td>21.9d</td></tr>
</table>
<u>Metabolites:</u>
DTPU
DTPP
HTPP
TPSA</td>
<td>The study is considered acceptable.

Kinetic evaluation of the raw data from Anonymous (1996).</td>
<td>Anonymous (1996)

Anonymous (2014).</td>
</tr>
<tr>
<td>Aqueous Photolysis of Flazasulfuron under laboratory conditions.</td>
<td>Degradation of [^{14}C]SL-160 was observed in pH 7 buffer solutions, in both the treated irradiated and dark control samples.</td>
<td>The study is considered acceptable.</td>
<td>Anonymous (2014).</td>
</tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>Guidelines:
OECD 216</td>
<td>
<table>
<tr><th>Sample</th><th>DT_{50} [days]</th><th>DT_{90} [days]</th></tr>
<tr><td>Irradiated samples</td><td>16.5</td><td>54.9</td></tr>
<tr><td>Dark control samples</td><td>5.7</td><td>18.8</td></tr>
</table>

<u>Metabolites:</u>
Irradiated samples
DTPU (SSRE-004)

Dark samples
DTPU (SSRE-004)
DTPP (SSRE-005)
</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous Photolysis of Flazasulfuron under laboratory conditions.

Guidelines:
Not indicated.</td>
<td>At pH 7, Flazasulfuron is initially stable to photolysis. Hydrolysis is the mechanism of reaction for the first 7 days.

Max. 2.5 % mineralisation after 90 days.

14C-Flazasulfuron (P):
<table>
<tr><th>Date Range</th><th>DT_{50}(Days)</th></tr>
<tr><td>0-30days</td><td>8.9</td></tr>
<tr><td>0-7 days</td><td>17.1</td></tr>
<tr><td>7-30 days</td><td>8.0</td></tr>
<tr><td>Hydrolysis Control</td><td>17.3</td></tr>
</table>
14C-Flazasulfuron (Pm):
<table>
<tr><th>Date Range</th><th>DT_{50}(Days)</th></tr>
<tr><td>0-30days</td><td>8.0</td></tr>
<tr><td>0-7 days</td><td>15.5</td></tr>
<tr><td>7-30 days</td><td>7.0</td></tr>
<tr><td>Hydrolysis Control</td><td>15.9</td></tr>
</table>
</td>
<td>The study is considered acceptable.</td>
<td>Anonymous (1995).</td>
</tr>
</table>

11.1.1 Ready biodegradability

Author(s): Anonymous (2014).

Title: Ready Biodegradability of sl-160 TGAI in a Manometry Respirometry Test.

Guidelines: OECD No. 301

GLP: Yes

This study determinated the biodegradability of Flazasulfuron over a period of 28 days in a manometric respirometry test. A sample of aerobic activated sludge collected from a sewage treatment plant, which treats predominantly domestic wastewater, and prepared in accordance with the guideline, was dosed with Flazasulfuron and incubated for 28 days at 22ºC in duplicate and test concentrations of 103 and 166 mg/L. Evolved carbon dioxide was trapped in an aqueous solution (45%) of potassium hydroxide. The consumption of oxygen was determined each day by measuring the change of pressure in the flasks by means of a manometric method (BSB/BOD-Sensor-System, Aqualytic Dortmund, Germany). The test flasks were closed gas-tight by a measuring head.

Findings:

The test item SL-160 TGAI contains nitrogen, thus the evaluation of biodegradation has to be based on ThODNH4 and ThODNO3 if the test item is biodegradable. Until day 28, 0 % of the applied SL 160 TGAI was biodegraded. As biodegradation did not reach the pass criterion of 60 % degradation and no 10 day-window could be determined, the validity criterion was not applicable. At the end of the experiment, the difference in replicate variation was 0 %.

The oxygen demand in the abiotic control was 0 mg/L during the test period. Therefore it was not necessary to correct the degradation of the test item and toxicity control.

The pH-value of the test item flasks at the end of the test was 7.4 and thus within the range of pH 6.0 to 8.5 as required by the test guideline.

Conclusion:

Within the test, the oxygen consumption in mixtures containing SL 160 TGAI never exceeded those of the control mixtures. Since substances are considered to be readily biodegradable in this type of test if oxygen consumption is equal to or greater than 60% of the theoretical value within ten days of the level achieving 10 %, SL-160 TGAI was not considered to be readily biodegradable under the conditions of this test.

The results obtained for the rate of degradation of sodium benzoate (85 % degradation after 28 days) and for the cumulative amount of oxygen consumed by the control mixtures (0 mg O2/L during test period) fulfilled the validity criteria for this test.

In the presence of SL 160 TGAI, the degradation of the reference item sodium benzoate achieved 66 % after 28 days indicating that the test substance was not inhibitory to the microbial inoculum.

The study is considered acceptable.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

One study to address the data requirement of hydrolytic degradation (Anonymous, 1995) was included in the submission for Annex I inclusion under Directive 91/414/EEC and was deemed acceptable following evaluation and peer review at EU level (1999). However, the kinetic of this study have been re-assessed (Anonymous, 2014) according to FOCUS degradation kinetics guidance (2006, 2011). The summary of this re-assessment has been included below.

Author(s): Anonymous (1995)

Title: A hydrolysis study of SL-160 in water.

Guidelines: Not indicated

GLP: Yes

The objective of this study was to investigate the rate and route of hydrolysis of SL-160 (Flazasulfuron). SL-160 labelled in the pyridine ring, [^{14}C]SL-160(P), or in the pyrimidine ring, [^{14}C]SL-160(Pm), was added to sterile buffered solutions at pH 4, 5, 7 and 9. The concentrations of the test substances were approximately 2 µg/mL and acetonitrile (<1%) was used as cosolvent. These solutions were maintained in the dark at 22 ± 1ºC or at 37 ± 1ºC. At selected intervals, samples were analysed directly by high-performance liquid chromatography with radiochemical flow detection (radio-HPLC).

Sample time varied with pH, label position and temperature. At 22ºC, samples were taken at zero time then at least 7 additional times between Day 1 and Day 30.

At 37ºC, a minimum of 24 samples were taken between 0.19 and 12 hours at pH 4; a minimum of 17 samples between 0.45 and 58 hours at pH 5; a minimum of 21 samples between 0.29 and 231 hours at pH 7; and a minimum of 14 samples were taken between 0.5 and 145 hours at pH 9.

At selected intervals, samples were analysed by high-performance liquid chromatography with radiochemical flow detection (radio-HPLC).

Findings:

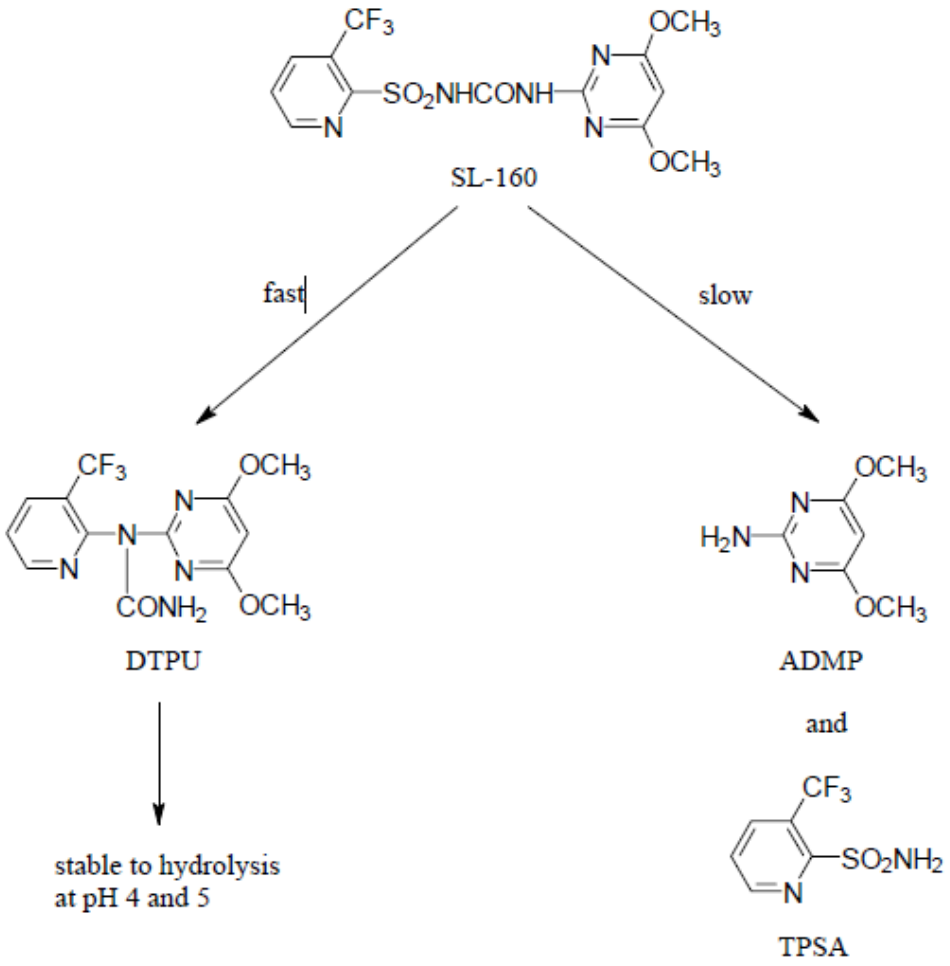
At pH 4, 5 and 7 and at 22ºC, the main product of hydrolysis of both ^{14}C-SL-160 (P) and ^{14}C-SL-160 (Pm) was DTPU. A minor product (up to approximately 10%) was DTPP. The levels of TPSA from ^{14}C-SL-160 (P) and ADMP of ^{14}C-SL-160 (Pm) slowly increased with time at pH 4 and 5. At pH 7, TPSA and ADMP were minor products of hydrolysis (<5%).

At pH 9 and at 22ºC, the major product of hydrolysis of both ^{14}C-SL-160 (P) and 14C-SL-160 (Pm) was DTPP. DTPU reached a maximum at Days 7 to 14 and then decreased with time. At pH 7, the

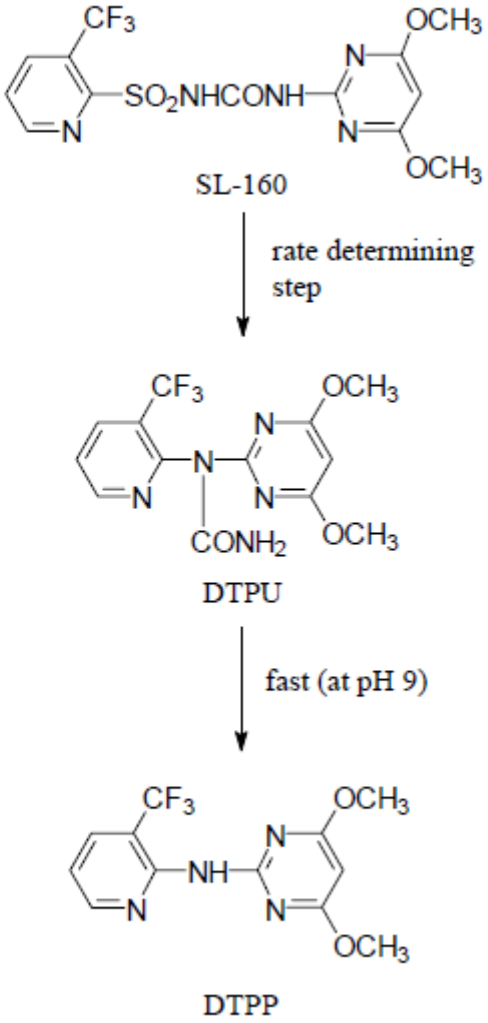
amounts of TPSA from ^{14}C-SL-160 (P) and of ADMP from 14C-SL-160 (Pm) never exceeded approximately 5% of the radiolabel. In all cases, the recovery of radioactivity was essentially quantitative.

The same products of hydrolysis were detected at 37ºC as at 22ºC, but a quantitative distribution was not determined at any pH or at any time point.

Mechanism of hydrolysis at pH 4, 5 and 7.



Mechanism of hydrolysis at pH 9.



Conclusion:

DTPU is the major product of hydrolysis at pH 4, 5 and 7. DTPP is the major product of hydrolysis at pH 9. TPSA and ADMP are minor products at pH 4 and 5 from 14C-SL-160 (P) and 14C-SL-160 (Pm), respectively.

The study is considered acceptable.

Author(s): Anonymous (2014)

Title: Kinetic evaluation of water/sediment and hydrolysis studies of Flazasulfuron and its metabolites according to FOCUS degradation kinetics guidance related to the supplementary dossier.

Guidelines: FOCUS Degradation Kinetics (2006, 2011).

GLP: No

Degradation of Flazasulfuron and its metabolites DTPU, TPSA, DTPP, HTPP and ADMP during hydrolysis in water systems were analysed according to the FOCUS Guidance on Degradation Kinetics (FOCUS, 2011)

to derive modelling DT_{50} values for these compounds. The analysis of the data from the study was conducted with the software KinGUI 2.1 as well as on the basis of visual assessment of the degradation curve.

The hydrolysis study (Anonymous, 1995) included four pH values (4, 5, 7 and 9) with labelled active substance (P- and Pm-label), whereas only the two pH systems 5 and 7 with relevance for environmental risk assessment (PECsw modelling) were considered during this evaluation.

Findings:

For the P-labelled SL-160 DT_{50} values, calculated by Single First Order kinetics (SFO), were 3.68 and 16.0 days at pH 5 and pH 7, respectively. For the Pm-labelled SL-160, DT_{50} values of 3.44 and 15.7 days were derived by SFO for pH 5 and 7.

	pH 5		**pH 7**	
	DT_{50}	**DT_{90}**	**DT_{50}**	**DT_{90}**
	P-label			
Flazasulfuron	3.68	12.2	16	53.2
TPSA	>1000	>1000	>1000	>1000
DTPU	382	>1000	70.3	233
DTPP	>1000	>1000	37.7	125
	Pm-label			
Flazasulfuron	3.44	11.4	15.7	52.3
ADMP	>1000	>1000	110	364
DTPU	425	>1000	87.3	290
DTPP	>1000	>1000	>1000	>1000

11.1.4 Other convincing scientific evidence

No data available

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Aerobic mineralisation.

A new study (Anonymous (2014)) was submitted for the EU review on the aerobic mineralisation of Flazasulfuron in surface water. The study followed OCED guideline 309 (November 2004) and was conducted to GLP. A summary is provided below, with a robust summary provided in Annex I of this dossier.

Author(s): Anonymous, (2014).

Title: SL-160: Aerobic mineralisation in surface water (pelagic test).

Guidelines: OECD Guideline 309

GLP: Yes

The aerobic degradation of SL-160 was studied in surface water sampled from a river in the UK. [^{14}C]SL-160 was applied (0.002 and 0.010 mg/L, respectively), [pyrimidine 14C]- and [pyridine 14C] labelled. Samples were incubated for 90 days. The systems were maintained in darkness at approximately 20ºC.

For each radiolabeled form of the test substance and each concentration, single replicates of surface water were taken for analysis immediately after application and after 2, 7, 10, 16, 24, 31, 43, 50, 70 and 90 days of incubation.

Chromatographic analyses were performed for surface water samples that received [^{14}C]SL-160; analyses were conducted with HPLC and TLC.

At the beginning and end of the incubation period, samples of surface water were analysed separately for aerobic bacteria, aerobic bacterial spores, actinomycetes and fungi.

Findings:

Validity of test determination:

The total recoveries of radioactivity in the surface water treated with reference substance [^{14}C]-sodium benzoate (at a nominal concentration of 0.010 mg/L) were 97.1-99.4 % AR after 14 days of incubation. Direct volatile radioactivity, almost all associated with $^{14}CO_2$, accounted for 78.5 to 86.8 % AR after 90 days. Therefore, the test was shown to be valid as the reference substance degraded within the expected time interval.

Samples for direct $^{14}CO_2$ determination

The total recoveries of radioactivity (i.e. the sum of radioactivity in the water layer and volatile radioactivity) ranged from 96.5 to 111.4 % AR.

After acidification to strip off any $^{14}CO_2$ from the samples treated at 0.002 mg/L, the total radioactivity in the surface water remained in the range 96.5 and 104.9 % AR throughout the 90 day incubation period. No direct volatile radioactivity ($^{14}CO_2$) was detected after 90 days.

After stripping of any $^{14}CO_2$ from the samples treated at 0.010 mg/L, the total radioactivity in the surface water remained in the range 101.4 and 111.4 % applied radioactivity throughout the 90 day incubation period. Direct volatile radioactivity ($^{14}CO_2$) accounted for a maximum of 0.6 % applied radioactivity after 90 days.

In the sterile surface water, the initial total recoveries of radioactivity in vessels treated with 0.002 mg/L and 0.010 mg/L of SL-160 were between 105.3-111.5 % AR after 90 days. No $^{14}CO_2$ was recovered in the trapping solutions.

Samples for chromatographic analysis

The total recoveries of radioactivity from samples ranged from 96.3 to 112.8 % AR.

HPLC analysis revealed the formation of up to two metabolites during degradation of SL-160. In samples with [^{14}C-pyrimidine]-SL-160, the amount of SL-160 in the surface water declined from 103.9 % AR (time zero) to 13.1 % after 90 days of incubation for samples treated with 0.002 mg/L and from 106.2% AR (time zero) to 13.2% AR after 70 days of incubation for samples treated with 0.010 mg/L.

The metabolite SSRE-004 (DTPU) reached a maximum of 53.4% AR (application of 0.002 mg/L) and 62.3% AR (application of 10µg/L) after 90 days. The metabolite SSRE-005 (DTPP) was detected in a concentration up to 90.6% AR and 45.3% AR in samples with low and high concentrations of SL-160, respectively.

In samples with [^{14}C-pyridine]-SL-160, the amount of SL-160 in the surface water declined from 98.3 % AR (time zero) to 14.1 % (day 90) and from 104.1 % to 16.7 % AR (day 70) in samples treated with 0.002 and 0.010 mg/L SL-160, respectively.

SL-160 was degraded to SSRE-004 (up to 48.1 % AR) and SSRE-005 (up to 79.0 % AR) after 90 days after application of 0.002 mg/L SL-160. When 0.010 mg/L SL-160 was applied, up to 61.6 % AR SSRE-004 and 47.0 % AR were measured after 90 days of incubation.

DT_{50} and DT_{90} values for SL-160 were calculated using the Single First-Order (SFO) kinetic model and the software Model Maker (version 4.0)

Concentration [µg/L]	**Kinetic model**	**DT_{50} [days]**	**DT_{90} [days]**	**Chi^2**
2	SFO	21	70	14.3
10	SFO	25	84	7.3

Conclusions:

Upon addition of [^{14}C]SL-160 to surface water, SL-160 degraded to less than 15 % AR until the end of the incubation period (90 days).

During incubation, two metabolites were detected. In the test systems with [pyrimidine ^{14}C]SL-160 and [pyridine ^{14}C]SL-160, the metabolites SSRE-004 (DTPU) and SSRE-005 (DTPP) were measured. DTPU reached a maximum of 62.3 % AR (day 43) and DTPP a maximum of 90.6 % AR (day 90).

Water/sediment studies.

Regarding water/sediment system, one study (Anonymous 1996) was included in the submission for Annex I inclusion under Directive 91/414/EEC and were deemed acceptable following evaluation and peer review at EU level (1999).

In addition, a kinetics assessment (Anonymous, 2014) was performed in accordance with FOCUS degradation kinetics guidance (2006, 2011) on the raw data generated from Murray, M.; (1996) study.

Summaries of these studies are presented below, with robust summaries presented in Annex 1 of this dossier.

Author(s): Anonymous (1996)

Title: [^{14}C]-SL-160 degradation and fate in water/sediment system.

Guidelines: BBA Guideline Part IV; EPA Guidelines 162-4; SETAC-Europe Guidelines (March 1995); Japan MAFF Guidelines.

GLP: Yes

The objective of this study was to determine the rate and pattern of [^{14}C-P]SL-160 degradation and metabolism in water and in hydrosoil under aerobic aquatic conditions. The test substance was applied to the water phase at concentration of 0.33 µg/mL (300 ppb) or related to sediment: 0.33 mg/kg (based on the volume of water above the sediment surface).

At each sampling interval, the Oxidation Reduction Potential (ORP) and pH was measured in the water phase of the test system. Aerobic conditions were maintained throughout the study.

There were two systems, one at pH 6.6 and another at pH 7.7. For the Day 0, the duplicate test vessels containing water only were taken for each water/sediment type. For each water/sediment type, one test vessel was taken according to the following schedule: 0.08 (~2 hours), 0.25 (~6 hours), 1, 2, 3, 7, 10, 14, 21, 30, 60 and 100 days after test substance application. In addition, for the pH 6.6 test system, samples were taken on Days 61 and 72.

Radioactivity in the water phases was quantified by LSC. The composition of the radioactivity in the water and sediment phases and also the solids remaining in the test vessel after extraction of sediment was determined by HPLC

Findings:

Mass balance

The mass balance of the applied radioactivity was determined as the sum of the radioactivity found in the water phase, sediment extracts, volatile and bound radioactivity. This recovery was greater than 90% in both water/sediment systems at all sampling points. In the pH 7.7 water/sediment system the mass balance ranged from 92.5 to 106.3% (mean = 97.6%). In the pH 6.6 system values ranged from 91.0 to 98.2% (mean = 94.7%). These mass balances are within the acceptable guidelines of 90 - 110% of the applied radioactivity.

Distribution of Radioactivity

Water phase

The radioactivity in the water phase decreased steadily after Day 1 in both test systems, reaching minimum levels of 24.4% and 20.1% by Day 100 in the pH 7.7 and pH 6.6 systems, respectively.

- pH 6.6 system: SL-160 declined from 98.5% at Day 0 to 4.9% by Day 72, and was completely degraded by Day 100.
- pH 7.7 system: SL-160 declined steadily from 98.4% at Day 0 to 4.2% by Day 100 in the pH 7.7 system.

DTPU was the major degradation product in the water in both sediment/water systems. TPSA was present in the water phase at all sampling points in both test systems. HTPP was also formed in the water phases of both systems after 3-4 weeks of incubation.

Sediment phase

The ^{14}C levels in the sediment extracts generally increased over time in both water/sediment systems.

- pH 6.6 system: the level of applied ^{14}C in the extract increased from 1.5% at 2 hr to 44.5% by Day 100. SL-160 residues increased from 1.3% of the applied dose at 2 hr to a maximum of 15.7% at Days 10 and 21, before dropping to 6.3% by Day 100.

- pH 7.7 system: the level of applied radioactivity in the extract increased from 1.5% at 2 hr to 48.3% by Day 100. SL-160 residues increased from 1.4% at 2 hr to 11.7% by Day 30, then declined to 5.2% by Day 100.

DTPU was the major degradant in the sediment of both test systems through Day 30, after which point HTPP became the major degradation product. TPSA was present at low levels in the sediments of both systems over the course of the study.

Post-extraction solids (PES)

Radioactive residues in the PES (bound residues) increased throughout the study in both water/sediment systems.

- pH 6.6 system: PES residues rose from 0.04% at 2 hr to 25.8% by Day 100.
- pH 7.7 system: PES increased from 0.03% at 2 hr to 23.6% by Day 100.

The Day 100 PES samples from both sediment/water test systems were subjected to more rigorous extraction conditions in order to release some of the bound radioactivity. HPLC analysis of the extract from each system showed that samples contained a number of components, each representing less than 3% of the applied dose.

Volatile radioactivity

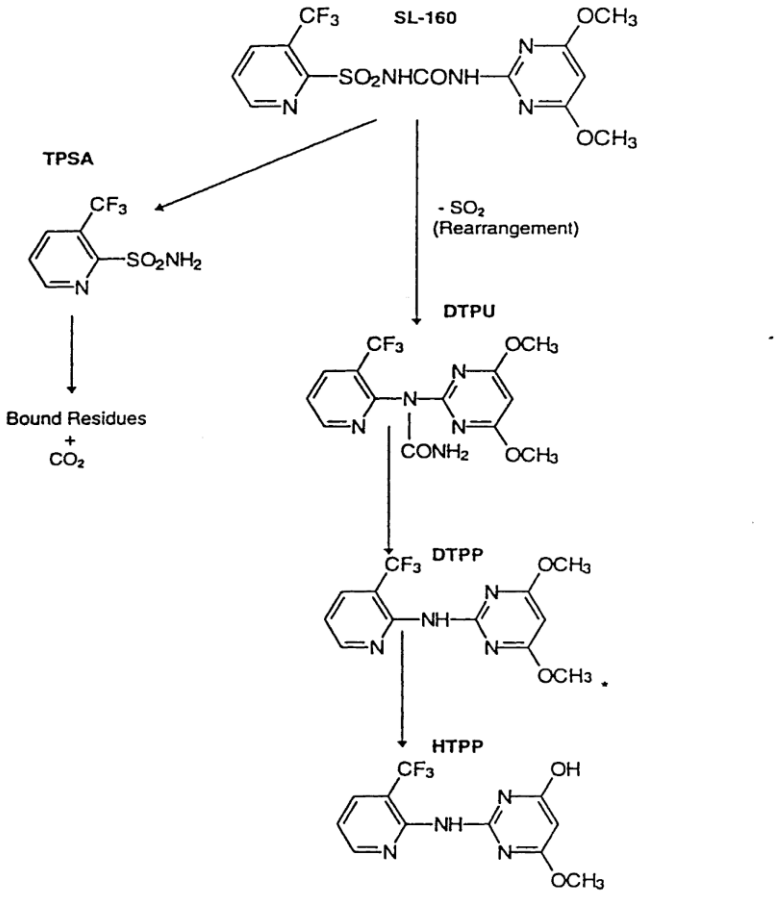
The level of radioactivity in the 1.0N NaOH traps as $^{14}CO_2$ was less than 1% in both sediment/water systems throughout the course of the study.

- pH 6.6 system: $^{14}CO_2$ was first detected on Day 1, increasing to 0.65% of the applied dose by Day 100.
- pH 7.7 system: $^{14}CO_2$ residues were first detected on Day 14, rising only to 0.28% of the applied dose by Day 100.

Radioactivity in the Carbotraps™ remained below 0.1% of the applied dose for the entire study in both test systems.

<u>Degradation pathway</u>

The proposed metabolic pathway for the degradation of SL 160 in aerobic water/sediment systems is shown in the following schematic.



Conclusions:

DTPU was the major degradation product detected in the water phase of each sediment/water system for the duration of the study. In the sediment extracts, DTPU was the major degradant in both systems through Day 30, but by about Day 60 HTPP levels had eclipsed those of DTPU, again in both test systems. Bound residues increased steadily in both systems over the 100-day sampling period, while parent SL 160 residues declined. Emission of radiolabeled volatiles, including $^{14}CO_2$, was minimal in both test systems throughout the study.

The kinetics of this study have been re-assessed according to FOCUS degradation kinetics guidance (2006; 2011) and normalized to reference conditions (see below Anonymous, 2014).

Author(s): Anonymous (2014)

Title: Kinetic evaluation of water/sediment and hydrolysis studies of Flazasulfuron and its metabolites according to FOCUS degradation kinetics guidance related to the supplementary dossier.

Guidelines: FOCUS degradation kinetics guidance (2006, 2011).

GLP: No

Degradation of Flazasulfuron and its metabolites DTPU, TPSA, DTPP, HTPP and ADMP during hydrolysis and in water/sediment systems were analysed according to the FOCUS Guidance on Degradation Kinetics

(FOCUS, 2011) to derive modelling DT_{50} values for these compounds. The analysis of the data from two studies was conducted with the software KinGUI 2.1 as well as on the basis of visual assessment of the degradation curve.

The water/sediment study (Anonymous, 1996) covered two systems of different pH values (6.6 and 7.7) treated with labelled SL-160 (P-label).

The kinetic assessment of the data from the water/sediment study was conducted at the first level for the parent substances according to the FOCUS report (PI level), i.e. degradation of the whole system and dissipation kinetics (decline) without consideration of exchange between compartments water and sediment. In the next step, the data was subjected to PII level evaluation under consideration of an exchange between the two compartments. For the metabolites, whole system formation and degradation kinetics as well as the decline in the water phase (MI level = one compartmental approach) were determined in conjunction with the parent compound and DT_{50} values were derived. Since mainly the formation phase of metabolites was covered during the study, a separate dissipation evaluation has not been applied, because of insufficient decline data points.

The calculations presented in the summary of the kinetic analysis of the w/s-study by Murray are based on Single First-Order (SFO) kinetics. SFO was statistically appropriate and showed suitable fits for all calculations regarding the parent, which dissipated to at least ca. 90% at the end of the experiments. With regard to the fit of the metabolite data, calculations where the parent was set to First-Order Multi-Compartment FOMC kinetics (used due to slightly better Chi² in calculations for the parent alone), showed slightly worse fits with regard to the metabolites, at least for DTPU and partly HTPP. In general, the differences in statistical parameters and visual fits were considered negligible between the different models used. In the light of the FOCUS models using SFO kinetics to simulate the dissipation, the selection of SFO was considered to be fully justified in all cases.

Rapporteur Member State commented:

Kinetics for parent only level I accepted for both systems. Level II kinetics for water phase and sediment are not considered reliable.

Kinetic for metabolites:

System with pH 7.7: it is noted that no decline at the end of the study is observed for metabolites HPTT and TPSA and this is reflected in the statistics and Chi² error (>20%) and the kinetics parameters for these metabolites are not considered reliable. RMS accepts the proposal for DTPU.

System with pH 6.6: it is noted that no decline at the end of the study is observed for metabolites HPTT, TPSA and DTPU, and this is reflected in the statistics of the estimated kinetics parameters and Chi² error (>20%), and they are not considered reliable.

RMS considers that the only reliable parameter which can be derived to be used in FOCUS modelling are:

DT_{50} values.

	pH 6.6	pH 7.7
	Whole system	Whole system
Parent	23.9 d	23.9d
DTPU	-	21.9d

11.1.4.4 Photochemical degradation

There are two studies on photochemical degradation under laboratory conditions (Anonymous, 2014 and Anonymous, 1995). They are summarised below.

Authors: Anonymous 2014

Title: SL-160 photodegradation in water and determination of the quantum yield.

Guidelines: OECD 216.

GLP: Yes

The photodegradation of SL 160 was studied in sterile pH 7 buffer solution at a nominal application rate of 10 mg/L. [^{14}C]SL 160 was applied either as [pyrimidine-^{14}C] or [pyridine ^{14}C] labelled test item. Samples were incubated for a period of 12 days.

For the main experiment, solutions treated with [^{14}C]SL-160 were irradiated with a xenon arc light source and filters to cut off light of less than 290 nm wavelength. Samples were irradiated at 25 ± 2 °C for 12 days which was equivalent to 28-29 days of natural summer sunlight at a latitude of 40 N. Dark control samples were prepared as irradiated samples but not submitted to irradiation. Solutions were analysed by LSC, HPLC and TLC.

Samples were taken for analysis immediately after application of SL-160 as well as 1, 3, 4, 7, 10 and 12 days after test substance application.

Findings:

Mass balance ranged from 98.8 to 100.3 % AR In the irradiated samples, volatile radioactivity in the potassium hydroxide traps reached a maximum of 0.5% AR after 12 days of irradiation and was considered to be associated with $^{14}CO_2$.

Degradation of [^{14}C]SL-160 was observed in both the treated irradiated and dark control samples.

In the irradiated buffered water samples, the amount of SL-160 declined from 97.4 98.9 % AR (day 0) to 56.6 64.5 % AR (day 12). SL 160 degraded to SSRE-004 (up to 28.3 % AR), SSRE-005 (up to 1.4 % AR), and up to ten unknown metabolites in the course of the experiment. The unknown metabolites were present at levels of ≤4.1 %.

In the non-irradiated control samples, significant degradation of SL-160 was observed. The amount of SL-160 in samples treated with [14C]SL-160 declined to 20.6-24.2 % AR (day 12). SL-160 degraded to SSRE-004 (up to 58.3 % AR), SSRE 005 (up to 14.9 %), SSRE-003 (up to 0.3 %) and up to three unknown metabolites in the course of the experiment.

DT_{50} and DT_{90} values for SL-160 were calculated using Single First Order (SFO) Kinetic model.

Sample	**Degradation rate k**	**DT_{50} [days]**	**DT_{90} [days]**
Irradiated samples	0.042	16.5	54.9
Dark control samples	0.122259	5.7	18.8

As the degradation rate of SL 160 was faster in the dark control samples than in the irradiated sample it was concluded that no photolysis occurred for SL-160 under the conditions of this test

The quantum yield was not determined as no photolysis was observed for SL 160.

Conclusions:

Degradation of SL 160 in pH 7 buffer was faster in dark control samples (DT_{50} 5.7 days) than in irradiated samples (DT_{50} 16.5 days), indicating that no photolysis occurred at test conditions. Major photodegradation products of SL 160 were SSRE-004 (irradiated and dark control samples) and SSRE-005 (dark control samples).

The study is considered acceptable.

Author(s): Anonymous. (1995).

Title: A photolysis study of SL-160

Guidelines: Not indicated.

GLP: Yes

The objective of this study was to investigate the rate and route of photolysis of SL-160 (Flazasulfuron) in water at pH 7.

SL-160 labelled in the pyridine ring (^{14}C-SL-160 (P)), or in the pyrimidine ring (^{14}C-SL-160 (Pm)), was added to sterile buffered solutions at pH 7. The concentrations of the test substances were approximately 0.002 mg/mL and acetonitrile (<1%) was used as cosolvent. The solutions were maintained at 22±1ºC either in the light or in the dark.

Samples were taken at zero time and then at Day 1, 3, 7, 10, 14, 21 and 30. At selected intervals , samples were analysed directly by HPLC with radiochemical flow detection.

Findings:

Recovery of ^{14}C from the dark control (hydrolysis) and light-exposed (photolysis) samples is summarized in the table below.

Recovery of ^{14}C from the HPLC Analyses

	Hydrolysis (dark simples)		Photolysis (irradiated simples)	
	^{14}C-SL-160 (P)	**^{14}C-SL-160 (Pm)**	**^{14}C-SL-160 (P)**	**^{14}C-SL-160 (Pm)**
Inject[1]	96.4%	95.9%	103.9%	97.6%
Collect[2]	95.8%	94.9%	102.3%	95.5%
Tag[3]	95.2%	93.7%	102.6%	95.6%

[1] The ^{14}C in each sample aliquot injected into the HPLC
[2] Total ^{14}C collected in the effluent from each HPLC analysis
[3] Total ^{14}C detected in the peaks by radio-HPLC

Distribution of ^{14}C in the dark control samples

The percentage of SL-160 in the ^{14}C-SL-160 (P) treated samples steadily decreased to 30.2% at Day 30. DTPU was the major product of hydrolysis at 57.3% of the total ^{14}C by Day 30. DTPU slowly converted to DTPP, with 9.3% of the total ^{14}C by Day 30.

The percentage of SL-160 in the ^{14}C-SL-160 (Pm) treated samples steadily decreased to 26.6% at Day 30. DTPU was the major product of hydrolysis at 60.1% of the total ^{14}C by Day 30. DTPU slowly converted to DTPP, with 9.5% of the total ^{14}C by Day 30.

Distribution of ^{14}C in the light-exposed samples

The percentage of SL-160 in the ^{14}C-SL-160 (P) treated samples decreased to 10.5% at Day 30. DTPU was the first major product detected. The percentage of this component changed from 3.3% at Day 1 to 26.1% at Day 10 and then changed more slowly 39.9% at Day 30. Low percentages of DTPP (5.1%) and TPSA (4.9%) were also detected at Day 30.

After seven days, an unknown unstable photoproduct, or a complex mixture of polar products, accelerates the rate of photolysis of SL-160.

The percentage of SL-160 in the ^{14}C-SL-160 (Pm) treated samples decreased to 7.3 % at Day 30. DTPU was the first major product detected. The percentage of this component reached a maximum of 40.6% at Day 21 and then decreased to 35.5% at Day 30. A low percentage of DTPP was detected at Day 30.

Again an unknown unstable photoproduct was detected, and a complex mixture of polar products at Day 30.

Rates of photolysis in the light-exposed samples

The half-life and rate constant data for SL-160 are presented in the following table.

Half-lives for the Light-Exposed and Dark Control Samples

Date Range	**^{14}C-SL-160 (P) Half-Life (Days)**	**^{14}C-SL-160 (Pm) Half-Life (Days)**
0-30 days	8.9	8.0
0-7 days	17.1	15.5
7-30 days	8.0	7.0
Hydrolysis Control (dark)	17.3	15.9

A linear regression analysis of ln(F), the fraction of total ^{14}C in solution coeluting with SL-160, versus time was used to evaluate half-lives. The slopes derived from the linear regression analysis were used to calculate half-lives.

The data indicate that SL-160 is initially stable to photolysis and the hydrolysis is the mechanism of reaction for the first seven days. After seven days, the photoproduct or a component in the polar fraction accelerates the rate of reaction.

Conclusions:

In water exposed to sunlight in the environment, the route of degradation of SL-160 is the initial hydrolysis to DTPU that accounts for about the 60 % of the initial Flazasulfuron after 30 days. This intermediate is slowly converted to DTPP with 9.5 % after 30 days. There appears an unknown photoproduct that reaches a maximum of 10.3 % after 14 days to decrease to 6.5 % after 30 days. Other polar not characterised metabolites account for the 33%.

Mineralization to carbon dioxide consumes only about 2.5% of the ^{14}C-SL-160 (Pm) label and none of the ^{14}C-SL-160 (P) label.

The study is considered acceptable.

Overall conclusions on degradation.

Flazasulfuron is considered not readily biodegradable according to the result of the biodegradation test presented (0 % biodegradation in 28 days)), following OECD 301 B guideline. The ready biodegradability criterion stated in this guideline considers substances readily biodegradable when 70% biotic degradation takes place in the 10 days window within the 28 days long duration test.

Flazasulfuron in not hydrolytically stable at pH values of 4, 5, 7 and 9 at 22ºC and 37ºC under sterile conditions in the dark for 30 days. After the kinetic evaluation of the raw data from Korsch (1995) the values of DT50 were:

	pH 5	**pH7**
DT50	3.44-3.68	15.7-16
DT90	11.4-12.2	52.3-53.2

In an aerobic mineralization study Flazasulfuron degraded with DT_{50} values of 21 and 25, depending on test concentration, to the following metabolites: DTPU and DTPP. Mineralisation reaches a maximum of 0.6% after 90 days.

Formation of metabolites is the main route of degradation of Flazasulfuron in water/sediment systems. DT50 values were calculated for the whole system (DT50 23.9d). And at the end of the water/sediment study, the maximum carbon dioxide was 0.65% of the applied dose (pH 6.6 system) indicating minimal mineralization.

Regarding photodegradation, degradation of Flazasulfuron was observed in both the treated irradiated samples (DT50 16.5d) and dark control samples (DT50 5.7d) indicating that hydrolysis is the mechanism of reaction. Photolysis is not expected to be a major route of degradation.

Due to the results summarized above, Flazasulfuron can be considered as a not rapidly degradable substance in the environment, according to the CLP criteria.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

Soil adsorption

In the Review Assessment Report, two studies on adsorption and desorption in soils were considered valid for Flazasulfuron (Anonymous, 2006 and Anonymous, 1996).

In Anonymous (1996) results show that Flazasulfuron was classified as very highly mobile (K_{OC} = 0-50) in three of the soils tested, and highly mobile (K_{OC} = 50-150) in the fourth soil tested. The average K_{OC} (for both data sets) is 46.16. Based on the K_{OC} values, flazasulfuron would be expected to show significant downward movement in soils.

In Anonymous 2006, Flazasulfuron was classified as high mobile in two of the soils tested, as medium mobile in the third soil; and as low mobile in soil the fourth soil tested.

There are also studies on adsorption and desorption in soils for metabolites that appear in water/sediment studies (TPSA, DTPU, DTPP, ADMP, HTPP) and also for GTPS metabolite (this metabolite presents a toxicity value for *Lemna gibba* less than 1 mg/L; see point 11.5, Acute aquatic hazard). These metabolites are considered between high mobile and medium mobile depending on the soil tested.

These results do not impact the conclusion regarding degradation as not rapidly according to CLP criteria and there are not summarised below although their robust summaries are presented in Annex 1 of this dossier.

Volatilisation.

Based on the vapour pressure (< 1.33×10^{-5} Pa, 25 °C) and low Henry´s law constant (2.5799 x 10^{-6} Pa m^3 mol^{-1} at 25ºC), Flazasulfuron is virtually non-volatile, therefore, significant exposure to air is not to be expected. However, there is a degradation study on Flazasulfuron by photo-oxidation (Vöekel, 1998) which was provided in the DAR (1999). The rate constant was estimated and it allowed to calculate the atmospheric half-life of Flazasulfuron: 0.63 hours, which corresponds to 0.053 days when a 12-hour day is considered and 0.027 days when a 24-hour day is considered. These results do not impact the degradation classification as not rapidly, and there are not summarised below although their robust summaries are presented in Annex 1 of this dossier.

11.4 Bioaccumulation

Table 111: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water Method: 40 CFR 158.190	log K_{ow} = 1.30 at pH 5 at 25 °C log K_{ow} < 0.06 at pH 7 at 25 °C Data not relevant at basic pH because Flazasulfuron is not stable under alkaline conditions (e.g., pH 9) under the study conditions used. Flazasulfuron is easily ionizable and will tend to remain in water at neutral or basic pH. The low mean K_{OW} at pH 7 indicates that at this pH, Flazasulfuron will not significantly partition into or tend to accumulate in living cells.	This study is considered acceptable.	Anonymous (1994)

11.4.1 Estimated bioaccumulation

As experimental data are available, estimations of bioaccumulation potential are not required.

11.4.2 Measured partition coefficient and bioaccumulation test data

Author(s): Anonymous (1994).

Title: Octanol/water partition coefficient of Flazasulfuron.

Guidelines: Method: 40 CFR 158.190, page 63 – 11.

GLP: Yes

This study aimed to determine the partition coefficient n-octanol/water of Flazasulfuron at 25ºC (purity 99.8%).

Findings:

Mean K_{OW} = 20 at pH 5 at 25ºC and is not concentration dependent at pH 5, the mean logK_{OW} = 1.30 at pH 5. Mean K_{OW} at pH 7 was well below 10 at 25ºC the mean log K_{OW} <-0.06 at pH 7.

Conclusions:

Data not relevant at basic pH because Flazasulfuron is not stable under alkaline conditions (e.g., pH 9) under the study conditions used. Flazasulfuron is easily ionizable and will tend to remain in water at neutral or basic pH. The low mean K_{OW} at pH 7 indicates that at this pH, flazasulfuron will not significantly partition into or tend to accumulate in living cells.

The study is acceptable.

With regard to bioaccumulation of Flazasulfuron, no studies are available to establish measured BCF estimates and log Kow data have been used to conclude on the potential for bioaccumulation of this substance. The log Kow values for Flazasulfuron is 1.3 at pH 5 and less than 0.06 for pH 7, which are less than the CLP log Kow trigger value of > 4 intended to identify substances with a potential to bioaccumulate under CLP criteria. Both log Kow estimates are lower than the CLP trigger value of 4 and, therefore, Flazasulfuron is considered to have low potential to bioaccumulate.

11.5 Acute aquatic hazard

A brief summary of the aquatic toxicity studies evaluated during Annex I inclusion of Flazasulfuron and submitted for the purposes of EU renewal is reported below. From all available ecotoxicity tests on this substance only information considered adequate, reliable and relevant for the classification proposal has been included.

The available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA, ADMP, HTPP) revealed toxicity values > 1 mg/L and they present low level of toxicity. Therefore, the studies with these metabolites are not described here in detail but they are included in Annex I to this dossier.

On the other hand, the toxicity data of metabolite GTPS revealed toxicity values to aquatic macrophytes (*Lemna gibba*) < 1 mg/L and the study with this metabolite is summarized below.

Table 112: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Acute toxicity to fish. US EPA FIFRA No. 72-1; ASTM Standard E 729 - 88	Rainbow trout (*Salmo gairdneri)*	Flazasulfuron technical (97.1% purity). The pH ranged from 8.0 to 8.4.	96h-LC_{50} = 22 mg/L, based on mean measured concentrations	The study is considered acceptable.	Anonymous, (1995).
Acute toxicity to fish. US EPA FIFRA No. 72-1; ASTM Standard E 729 - 88	Bluegill sunfish (*Lepomis macrochirus)*	Flazasulfuron (97.1% purity). The pH ranged from 7.8 to 8.2.	96h-LC_{50} > 98 mg/L based on mean measured concentrations	The study is considered acceptable.	Anonymous (1995).
Long term and chronic toxicity to fish. OECD 204	Rainbow trout (*Onchorhynchus mykiss*)	Flazasulfuron (95.7% purity). The pH ranged from 7.2 to 8.2.	21-d LC_{50} > 17 mg/L based on mean measured concentrations	The OECD TG 204 is not considered suitable anymore Supplementary information.	Anonymous (1995).
Acute toxicity to aquatic invertebrates. US EPA 540/9-82-024, US EPA	Cladoceran (*Daphnia magna)*	Flazasulfuron technical (97.1% purity). The pH ranged from 7.7 to 8.1.	48h-EC_{50} > 106 mg/L based on mean measured concentrations	The study is considered acceptable.	Anonymous(1995).

Method	Species	Test material	Results[1]	Remarks	Reference
540/9-85-006; ASTM E 729-88; FIFRA 72-2.					
Acute toxicity to algae or other aquatic plants. OECD Guideline 201	Green alga (*Pseudokirchneriella subcapitata)*	Flazasulfuron technical	72h-ErC_{50} = 0.074 mg/L, based on geometric mean concentration	The validity criteria were not met. Supplementary information.	Anonymous(1995)
Acute toxicity to algae or other aquatic plants. OECD Guideline 201; US EPA OPPTS 850.5400 (1996)	Freshwater green alga (*Pseudokirchneriella subcapitata)*	Flazasulfuron technical (98.96% purity). The pH ranged from 7.9 to 9.3.	72h-ErC_{50} = 0.0177 mg/L 72h-EyC_{50} = 0.00252 mg/L, based on nominal concentrations	The study is considered acceptable.	Anonymous. (2011).
Acute toxicity to algae or other aquatic plants. OECD 201; Directive 92/69/EEC, C3 (1992).	*Anabaena flos-aquae*	Flazasulfuron technical (97.3% purity). The pH ranged from 7.7 to 10.2.	EC_{50} value could not be calculated due to mathematical reasons. (72h-ErC_{50} > 0.075 mg/L).	No fulfilment of the validity criteria, and the impossibility to calculate an EC_{50} Supplementary information.	Anonymous(1996)
Acute toxicity to algae or other aquatic plants. US EPA 797.1160	Duckweed (*Lemna gibba)*	Flazasulfuron technical (97.3% purity) The pH ranged from 5.0 to 5.3.	7d-$E\mu C_{50}$ = 0.000058 mg/L .	There are some uncertainties related to analytical results and the validity criteria were not met Supplementary information.	Anonymous(1996)
Acute toxicity to algae or other aquatic plants.	Duckweed (*Lemna gibba)*	Flazasulfuron technical (99.7% purity)	**7d-$E\mu C_{50}$ = 0.0007 mg/L** (frond number)	The study is considered acceptable.	Anonymous(1999)

Method	Species	Test material	Results[1]	Remarks	Reference
OPPTS 850.4400; OECD 221		The pH ranged from 7.4 to 8.9 (part A).	based on mean measured concentrations		
Acute toxicity to algae or other aquatic plants. OPPTS 850.4400; OECD 221	Duckweed (*Lemna gibba)*	Flazasulfuron technical.	7d-EμC_{50} = 0.0011 mg/L, based on mean measured concentrations	This study was considered in the previous evaluation process but not included in the LoEP. Supplementaryinformation.	Anonymous(1999).
Acute toxicity to algae or other aquatic plants. Ring Test Protocol: "Rooted Aquatic Macrophyte, Myriophyllum spec." (2009), Ring Test Protocol: "Standardized method for investigating test substance impact on rooted aquatic macrophyte	*Myriophyllum aquaticum*	Flazasulfuron technical (98.5% purity). The pH ranged from 7.4 to 8.2.	7d-EμC_{50}(shoot length) > 0.0041 mg/L, based on mean measured concentrations	The validity criteria were not completely fulfilled. Supplementary information.	Anonymous (2014)
Acute toxicity to algae or other aquatic plants. OECD 239.	*Glyceria maxima*	Flazasulfuron technical (98.5% purity). The pH ranged from 7.8 to 9.6.	28d-ErC_{50} (shoot length)= 0.0242 mg/L based on mean measured concentrations.	The study is considered acceptable.	Anonymous (2014)
Acute toxicity to algae or other aquatic plants. OECD 221	Duckweed (*Lemna gibba)*	SSRE-018 (metabolite GTPS) (97.0% purity)	7d-ErC_{50} > 0.460 mg SSRE-018/L based on mean measured concentrations	The study is considered acceptable.	Anonymous (2014).

Method	Species	Test material	Results[1]	Remarks	Reference
		The pH ranged from 7.6 to 9.1.			

11.5.1 Acute (short-term) toxicity to fish

With regard to acute (short-term) toxicity to fish of Flazasulfuron, two studies were carried out. These studies were already evaluated during Annex I inclusion of Flazasulfuron and they were accepted.

Author(s): Anonymous. (1995).

Title: SL-160 Technical: Acute toxicity test in rainbow trout under flow-through conditions

Guidelines: The method followed the US EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation - Wildlife and Aquatic Organisms, Guideline No. 72-1; EPA Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish, and ASTM Standard E 729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians, which is comparable to 92/69/EEC Part B

GLP: Yes

The 96-hour LC_{50} of Flazasulfuron to rainbow trout (*Salmo gairdneri*) was assessed under continuous flow-through conditions. Five groups of rainbow trout (20 fish per group, 10 fish per test chamber) were exposed to nominal test concentrations of 6.5, 11, 18, 30 and 50 mg/L of Flazasulfuron. Another two groups of rainbow trout were exposed to either a solvent (Dimethyl formamide) control or a negative (well water) control.

Mortality and abnormal behaviour were recorded at 5.5, 24, 48, 72 and 96 hours after test initiation.

Findings:

The pH ranged from 8.0 to 8.4. Temperature in the test vessels ranged from 12.0 to 12.6 ºC throughout the experimental period.

Mean measured values taken at 0, 48 and 96 hours ranged from 102-120%, 103-127% and 104-126% of nominal, respectively. The 96-hour LC_{50} for Flazasulfuron was calculated to be 22 mg/L, based on mean measured concentrations. The NOEC was 8.1 mg/L and the LOEC value was 13 mg/L.

Author(s): Anonymous (1995).

Title: SL-160 Technical: Acute toxicity test in bluegill under flow-through conditions

Guidelines: The method followed the US EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation - Wildlife and Aquatic Organisms, Guideline No. 72-1; EPA Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish, and ASTM Standard E 729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians, which is comparable to 92/69/EEC Part B.

GLP: Yes

The 96-hour LC_{50} of Flazasulfuron to bluegill (*Lepomis macrochirus*) was assessed under flow-through conditions. Five groups of bluegill (20 fish per group, 10 fish per test chamber) were exposed to nominal test concentrations of 14, 24, 40, 66 and 110 mg/L of Flazasulfuron.

Samples were collected at 0, 48 and 96 hours to measure concentration of the test substance.

Mortality and abnormal behaviour were recorded at 4.5, 24, 48, 72 and 96 hours after test initiation

Findings:

The pH values ranged from 7.8 to 8.2 and temperatures varied between 21.9 and 22.1 ºC.

Mean measured values taken at 0, 48 and 96 hours ranged from 88-108%, 89-108% and 90-109% of nominal, respectively. The 96-hour LC_{50} for Flazasulfuron was higher than 98 mg/L, based on mean measured concentrations, and the NOAEC was 98 mg/L.

The next study is considered as prolonged acute study according to Guidance on Information Requirement and Chemical Safety Assessment Chapter R.7b: Endpoint specific guidance. Version 4.0, June 2017.

Author(s): Anonymous (1995).

Title: SL-160 Technical: 21-d prolonged toxicity study in the rainbow trout under flow-through conditions.

Guidelines: OECD 204.

GLP: Yes

The toxicity of Flazasulfuron to rainbow trout (*Onchorhynchus mykiss*) was assessed over a period of 21 days under flow-through conditions. 10 fish per each treatment and control group (mean length 50 mm and mean body weight 1.2 g) were tested as the nominal test concentrations of 0.078, 0.313, 1.25, 5 and 20 mg/L. The concentration of SL-160 Technical were measured at the beginning (day 0), on day 14, and on day 21. The test fish were observed for clinical sings on workdays and for mortality daily.

Findings:

No clinical sings were observed in the control tank and at all test concentrations.

The pH ranged from 7.2 to 8.2 during the test.

At the beginning of the exposure period (day 0), the concentrations in the test medium ranged from 97.2 % to 105.4 % of nominal, on day 14 from 76.1 % to 106.1 % of nominal and at the end of the 21-day exposure period from 72.1 % to 95.7 % of nominal.

Nominal concentration of Flazasulfuron (mg/L)	Time (days)	Mean measured concentrations (mg/L)	Percent of nominal	Geometric mean measured concentration
Control	0	ND	ND	ND
Control	14	ND	ND	

Control	21	ND	ND	
0.078	14	0.0828	106.1	0.077
0.078	21	0.0719	92.2	
1.25	14	1.038	83.0	0.987
1.25	21	0.9391	75.1	
5.0	0	4.858	97.2	4.840
5.0	14	4.879	97.6	
5.0	21	4.784	95.7	
20	0	21.08	105.4	16.663
20	14	15.22	76.1	
20	21	14.42	72.1	

[a] *The Day 0 samples for 0.078 and 1.25 mg/L could not be calculated due to interferences.*

It was noted that the mean measured concentrations fall below 80% in some test concentrations. Thus, it is considered that the results should be expressed in measured concentration:

21-d LC_{50} > 17 mg/L

21-d NOEC = 5 mg/L

The study was considered valid for Risk Assessment in the DAR 1999. However, as the OECD TG 204 is not suitable anymore the study is considered as supporting information

METABOLITES

The available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA, ADMP, HTPP) revealed toxicity values > 1 mg/L and they present low level of toxicity. Therefore, the studies with these metabolites are not described here in detail but they are included in Annex I to this dossier. In any case, these studies were undertaken according to OECD 203 and to GLP and are considered reliable.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

With regard to acute (short-term) toxicity to aquatic invertebrates of Flazasulfuron, one study was carried out (Martin, K. H., Shults, S., 1995). It was already evaluated during Annex I inclusion of Flazasulfuron and it was accepted.

Author(s): Anonymous (1995)

Title: SL-160 technical: Acute toxicity test in Daphnids under flow-through conditions.

Guidelines: US EPA 540/9-82-024, US EPA 540/9-85-006; ASTM E 729-88; FIFRA 72-2.

GLP: Yes

The acute toxicity of Flazasulfuron to *Daphnia magna* was determined under flow-through conditions. Five groups of daphnids were exposed to the test material over 48 hours. Exposure nominal concentrations were

14, 24, 40, 66 and 110 mg/L and each replicate solution was analysed for test material concentration at initiation (0 hours) and at 48 hours (termination) of the exposure period.

The daphnids were observed for mortality and signs of toxicity or abnormal behaviour at approximately 2.5, 24 and 48 hours after test initiation.

Findings:

The pH in alternate replicates of each treatment and control group was measured at 24-hours intervals. The pH values ranged from 7.7 to 8.1.

Samples collected on Day 0 had mean measured values that ranged from 92 to 106% of nominal values. Mean measured values for samples taken at 48 hours ranged from 93 to 106% of nominal. When measured concentrations of samples collected at initiation and termination were averaged, the mean measured concentrations for the study were 15, 22, 39, 61 and 106 mg/L.

Based on mean measured concentrations, the 48h-LC_{50} for Flazasulfuron in *Daphnia magna* was estimated to be greater than 106 mg/L, the highest concentration tested.

METABOLITES

The available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA, ADMP, HTPP) revealed toxicity values > 1 mg/L and they present low level of toxicity. Therefore, the studies with these metabolites are not described here in detail but they are included in Annex I to this dossier. In any case, these studies were undertaken according to OECD 202 and to GLP and are considered reliable.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

With regard to acute (short-term) toxicity to algae of Flazasulfuron, two studies were carried out. (Anonymous, 1995, and Anonymous, 2011). One of these studies (Anonymous, 1995) was already evaluated during Annex I inclusion of Flazasulfuron and it was accepted. For the purpose of EU renewal and according to Commission Regulation (EU) No 283/2013, a statistical re-analysis of data from Anonymous (1995) was presented. Taking into account this statistical re-analysis, the validity criteria were not met for this study and it is considered as supplementary data.

The other study (Anonymous, 2011) has been presented for the purpose of EU renewal of Flazasulfuron and it is considered valid.

Author(s): Anonymous (1995).

Title: Acute toxicity of SL-160 technical to *Pseudokirchneriella subcapitata*.

Guidelines: OECD Guideline 201; EEC Directive 92/69 C.3

GLP: Yes

The potential toxicity of Flazasulfuron to green algae (*Pseudokirchneriella subcapitata*) was investigated for 72 hours. Exposure nominal concentrations were in the range from 0.0097 to 0.5 mg/L. Samples of culture solutions were taken immediately prior to exposure and after 72 hours for analysis of test substance concentrations by HPLC.

Findings:

HPLC analysis showed at the beginning of the test (hour 0) concentrations of Flazasulfuron in the range from 92.9% to 100%. These HPLC analysis showed at the end of the test (after 72h) concentrations of Flazasulfuron in the range from 69.9% to 79.3%. Recoveries at the end of the test fell below 80% and it was considered that measured concentrations should be used to define endpoints.

The overall geometric mean recovery was 85%. In order to base the endpoint on measured concentrations it has been recalculated applying this overall geometric mean percentage (85%).

The end points proposed were:

72-h NOEC = 0.034x0.85= 0.029 mg SL-160/L

72-h ErC50 = 0.088x0.85= 0.075 mg SL-160/L

72-h EbC50 = 0.045 x 0.85=0.038 mg SL-160/L

Nevertheless, during the commenting period for the renewal of the substance, applicant was required to provide values of ECx (10, 20) together with the NOEC for all chronic/long term/reproductive toxicity studies, and this study was statistically re-evaluated in order to fulfil current data requirement (Commission Regulation (EU) No 283/2013).

Based on the results regarding growth rate, the 72-hour E_rC_{10}, E_rC_{20} and E_rC_{50} was determined to be 0.030, 0.041, and 0.074 mg a.s./L (geometric mean concentrations), and the 72-hour NOEC and LOEC were determined to 0.030 and 0.040 mg a.s./L (geometric mean concentration), respectively.

Based on the results regarding yield, the 72-hour E_yC_{10}, E_yC_{20} and E_yC_{50} was determined to be 0.020, 0.024, and 0.036 mg a.s./L (geometric mean concentrations), and the 72-hour NOEC and LOEC were determined to 0.018 and 0.030 mg a.s./L (geometric mean concentration), respectively.

	Growth rate after 72 hours [mg a.s./L]	Yield after 72 hours [mg a.s./L]
EC_{10}	0.030 (0.024 – 0.036)	0.020 (0.012 – 0.032)
EC_{20}	0.041 (0.034 – 0.049)	0.024 (0.016 – 0.038)
EC_{50}	0.074 (0.057 – 0.095)	0.036 (0.021 – 0.063)
NOEC	0.018	0.018
LOEC	0.030	0.030

Values in parenthesis: lower and upper 95 % confidence level

It should be noted that the LOEC from growth rate presented the same value than the EC10. Morever, the confidence intervals of EC_{10} and EC_{20} are overlapping, which increases the uncertainties about these results.

On the other hand, the mean coefficient of variation of the average specific growth rate during the entire test period in replicate control cultures is 49.2 % instead of 35% as the OCDE 201 guideline recommends. Therefore the validity criteria were not met for this study.

Taking into account the above consideration, it is difficult to consider the endpoints valid for the risk assessment. Therefore, the study is considered as supplementary information.

Author(s): Anonymous, (2011).

Title: Growth inhibition test of SL-160 technical with green algae (*Pseudokirchneriella subcapitata*).

Guidelines: JMAFF "Data requirements for supporting registration of Pesticides (2011); OECD 201; US EPA OPPTS 850.5400 (1996).

GLP: Yes

The effects of Flazasulfuron on the growth of the freshwater green algae (*Pseudokirchneriella subcapitata*) were determined under static conditions for 96 hours. Exposure nominal concentrations were 0.0010, 0.00222, 0.0046, 0.010, 0.022, 0.046 and 0.10mg/L. The test concentrations were verified by chemical analysis at 0, 72 and 96 hours, using HPLC with mass spectrometry.

Findings:

The pH at 0, 72 and 96 hours after the start of the exposure were 7.9-8.0, 7.9-9.3 and 7.9-8.8°C, respectively.

The temperature in the culturing apparatus during exposure period ranged within the nominal range of 22±2°C.

Since chemical analysis of the test solutions showed that measured concentrations throughout the study were within ± 20% of the nominal values, the results of the study are expressed in terms of nominal concentrations of the active ingredient.

Validity criteria

In the study the biomass of the control increased by a factor of 498 after 72 hours and was therefore above the validity criteria of 16 times. The mean coefficient of variation (CV) for section-by-section specific growth rates in the control replicates should not exceed 35 % and had been determined to be 6.6% for all control replicates. Also, the CV for the average growth rate of the control for the entire test period (0 to 72 h growth rate) should not exceed 7 % for *Pseudokirchneriella subcapitata* and was calculated to be 0.7%. Thus, all validity criteria had been fulfilled and the test can be regarded as valid.

After 96 hours of exposure, there were no signs of noticeable aggregation, flocculation, or adherence of cells to the test chambers in the negative control or in any treatment groups. There were no noticeable changes in cell morphology in any treatment levels when compared to the negative control.

Toxicity endpoints for growth rate and yield of *Pseudokirchneriella subcapitata* after exposure to SL-160 technical

	E_yC_{50} (95 % CI)	**NOE_yC**	**E_rC_{50} (95 % CI)**	**NOE_rC**
	[mg a.s./L]			
after 72 hours exposure	0.00252 (0.00227 – 0.00281)	0.0010	0.0177 (0.0164 – 0.0190)	0.0010
after 96 hours exposure	0.00342 (0.00319 – 0.00366)	< 0.0010	0.0217 (0.0201 – 0.0234)	0.0010

CI confidence interval

Additionally, EC_{10} and EC_{20} values were reported for growth rate.

EC_{10} and EC_{20} values for growth rate for *Pseudokirchneriella subcapitata* after exposure to SL-160 technical

	E_rC_{10} (95 % CI)	**E_rC_{20} (95 % CI)**
	[mg a.s./L]	
72 hours exposure	0.0018 (0.0014 – 0.0021)	0.0039 (0.0032 – 0.0047)
96 hours exposure	0.0033 (0.0027 – 0.0039)	0.0064 (0.0053 – 0.0074)

CI confidence interval

In addition to the above studies just presented, another study (Anonymous1996) conducted with additional algal species (*Anabaena flos-aquae*) had been submitted in order to evaluate toxicity of Flazasulfuron in other algae species. This study does not meet the validity criteria of OECD 201 Guideline needed for approval of active substances in the EU. Additionally, it was not possible to calculate an EC50. Therefore, this study is considered as supplementary information.

Author(s): Anonymous (1996).

Title: Toxicity of SL-160 technical to *Anabaena flos-aquae* (cyanophyta) in an algal growth inhibition test.

Guidelines: OECD 201; Directive 92/69/EEC, C.3 (1992).

GLP: Yes

The effects of Flazasulfuron on the growth of *Anabaena flos-aquae* (cyanophyta) were determined under static conditions for 96 hours. Exposure nominal concentrations were 0.001, 0.0032, 0.010, 0.032, and 0.1 mg/L. The test concentrations were verified by chemical analysis at 0, 72 and 96 hours.

Findings:

The pH of the test media were measured daily from all test concentrations at the start and end of the test. The pH at the start of the test for each of the test concentrations was within the range of 7.7 to 7.9. By the end of the test the pH had risen to between 9.6 and 10.2.

The temperature in the test media was between 24.3 °C and 25.7 °C during the testing period.

The analytically determined test substance concentrations in the sample from the freshly prepared stock solution amounted to 97% of the nominal value. In the samples from the freshly prepared test media, the mean measured test substance concentrations ranged from 58 to 59% of the nominal concentrations. After the test period of 96 hours, the concentrations of Flazasulfuron in the test media had further decreased to 46 to 50% of the nominal values. All reported biological results were therefore related to the mean measured test substance concentrations.

During the commenting period on the re-evaluation, new information regarding the fulfilment of the new relevant validity criteria was required by EFSA. Moreover, the applicant was also required to submit values of ECx and the NOEC for all chronic/long-term/reproductive toxicity studies.

Reviewing the information presented after EFSA requirement, the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is 1.8 % and therefore fulfils the validity

criteria OECD 201 (< 10%). However, the mean coefficient of variation for section-by-section specific growth rates is 43.8 % and therefore, the validity criteria according to OCDE 201 is not met (CV must not exceed 35 %).

ECx and NOEC calculations

For this study, no measurements were provided for the two lowest test levels (i.e. 1.0 and 3.2 µg a.s./L test concentrations). To obtain approximate geometric mean values for these test levels, an average of the geometric means for measured concentrations at 0 hours and 72 hours for each available treatment level was calculated. The geometric means ranged from 51.6 to 53.1% of nominal concentrations, averaging to an overall geometric mean of 52.5%. Therefore, all nominal test concentrations (1.0, 3.2, 10, 32, and 100 µg/L) were adjusted by 52.5% to 0.083, 0.53, 1.7, 5.3, 17, and 53 µg a.s./L.

	Growth rate after 72 hours [µg a.s./L]	Yield after 72 hours [µg a.s./L]
EC_{10}	36.8 (31.8 – 42.6)	10.0 (5.95 – 16.9)
EC_{20}	75.2 (59.6 -93.6)	16.8 (10.1 – 28.0)
EC_{50}	> 75.2	45.3 (22.8 – 86.3)
NOEC	5.3	5.3
LOEC	17	17

Values in parenthesis: lower and upper 95 % confidence level

RMS agrees with the applicant proposed data. However, it is noted that no EC_{50} value could be calculated due to mathematical reasons and therefore the applicant proposed to use $>EC_{20}$ as EC_{50} (EC_{50} > 75.2 µg/L).

Taking into account the no fulfilment of the validity criteria, and the impossibility to calculate an EC_{50}, RMS is of the opinion to consider the study as supplementary information.

METABOLITES

The available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA, ADMP, HTPP) revealed toxicity values > 1 mg/L and they present low level of toxicity. Therefore, the studies with these metabolites are not described here in detail but they are included in Annex I to this dossier. In any case, these studies were undertaken according to OECD 201 and to GLP and are considered reliable.

Effects on aquatic macrophytes.

Author(s): Anonymous (1996).

Title: Toxicity of SL-160 to the aquatic plant *Lemna gibba* G3 in a semi-static growth inhibition test.

Guidelines: US EPA 797.1160

GLP: Yes

The toxicity of Flazasulfuron to the aquatic plant *Lemna gibba* was determined in a 7-day semi-static test. Exposure nominal concentrations were 0.010, 0.032, 0.10, 0.32 and 10 µg/L and they were renewed after 3 and 6 days. Assessment of frond number were made on days 0, 3, 6 and 7.

The number of living and/or dead fronds was counted by use of a hand lens. Fronds visibly projecting over the edge of the mother frond were counted as separate fronds. Fronds that were completely yellowish colored were determined to be dead.

At the test termination the dry weight of all Lemna colonies in each test flask was determined.

The pH values of the test media were measured in all test concentrations and the control at the beginning and end of each treatment period. The water temperature was measured in one control flask each day. The air temperature in the temperature-controlled test room was continuously recorded.

The concentrations of Flazasulfuron technical were measured in all stock solution samples and in all duplicate test media samples from the nominal test concentrations 0.032 to 1.0 µg/L. From the control samples only 1 of the duplicate samples was analysed from each of both sampling dates (Days 0 and 3). The samples from the lowest test concentration of nominal 0.010 µg/L were not analysed since this concentration was below the determined 7-day NOEC.

Findings:

The pH measurements for each concentration and for each time interval were between a pH of 5.0 and 5.3. The temperature of the test media ranged from 24.9 to 25.6 °C.

There are some uncertainties in this study:

- The analytically results showed a high variability. Test substance concentrations in the samples from the freshly prepared stock solutions ranged from 48 to 84% of the nominal value and in the samples from the freshly prepared test media the mean measured test substance concentrations ranged from 41 to 128% of the nominal concentrations. After the longest test medium renewal period of 72 hours 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. Flazasulfuron technical was obviously not stable under the test conditions.

- **Analytical results**

Nominal test substance concentration	**Mean measured test substance concentration (average over all measurements per test conc.)**
0.010 µg/L	Not analysed
0.032 µg/L	0.02µg/L (= 64% of nominal)
0.10 µg/L	0.04 µg/L(= 40% of nominal)
0.32 µg/L	0.08 µg/L (= 25% of nominal)
1.0 µg/L	0.31 µg/L(= 31% of nominal)

- At the lowest test concentration of nominal 0.010 µg/L, the growth of *Lemna gibba* was statistically significantly reduced compared to the control at the observations times after 6 and 7 days. However, at the next higher test substance concentration of nominal 0.032 µg/L, the growth rate was not statistically significantly different from the control during the test period. The author considered that the reduced mean values of growth parameters at the test concentration of nominal 0.010 µg/L is unknown, but might be caused due to an irregular growth of the test plants at some of the test flasks after 6 or 7 days.

- Related to the validity criteria, it was not met. According to OCDE 221, the doubling time of frond number in the control must be less than 2.5 days (60 h), 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 this study was 3.0 days.

- Related to ECx calculation, the following results were presented by the applicant after EFSA requirement:

	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

Regarding growth rate, EC10 value is higher than the lower confidence interval of EC20, therefore, we cannot exclude that the estimation of EC10 could result in an effect much higher than 10 %. Moreover, the EC10 higher confidence interval is higher than the lower confidence interval of EC50. Therefore, ECx calculations are not reliable.

Due to related uncertainties this study is considered as supplementary information.

Author(s): Anonymous (1999).

Title: Toxicity of ^{14}C-SL-160 (P) to the aquatic higher plant *Lemna gibba* in a 7-day static growth inhibition test.

Guidelines: OPPTS 850.4400; OECD 221 draft guideline (Oct 2000).

GLP: Yes

The toxicity of Flazasulfuron to *Lemna gibba* was determined under static conditions for 7 days.

The test consisted of three parallel experimental parts:

- In experimental part A ("AAP-medium only") test plants were exposed in nutrient solutions with different test item concentrations as in a routine test. In this part the exposure nominal concentrations were 0.1, 0.2, 0.4, 0.8 and 1.6 µg/L and a control.
- In the experimental part B, the "20x AAP growth medium" used as in experimental part A. However sediment from a natural unpolluted pond was added.
- In the experimental part C, the test conditions were identical to those in experimental part B with exception that in experimental part C natural pond water was used instead of the synthetic growth medium.

  In the experimental parts B and C, exposure nominal concentrations were 0.8, 1.6 µg/L and a control.

The *Lemna* colonies in each test flask were inspected for changes in frond number and appearance (discoloration, sinking or other abnormalities) on Day 2 and 5 and at the end of the test (Day 7). The number of alive and dead fronds was counted. After the test termination the dry weight of all colonies was determined.

Inhibition of *Lemna* growth was determined from: a) the average specific growth rates µ for exponentially growing cultures, b) the area under the growth curves (AUC), and c) the final biomass determined on the basis of dry weight (DW).

The EC-values and NOEC/LOEC could be determined only for experimental part A.

Findings:

The pH-value of the test media in experimental part A ranged from 7.4 to 7.6 at the start and from 8.6 to 8.9 at the end of the test. In experimental parts B and C pH-values at the start of the test were 7.6 and 7.7, and at the test termination 7.7 and 7.3, respectively.

In the experimental part A 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.

Firstly, the following conclusions were accepted:

- Up to and including at least the nominal concentration 0.2 µg/L both growth parameters (area under the growth curve AUC and growth rate µ) were statistically not significantly lower than in the control. Since no toxic effect was observed at this concentration, the 7-day NOEC of SL-160 for *Lemna gibba* G3 was determined at the nominal concentration 0.2 µg/L.
- The area under the growth curve was statistically reduced after the exposure period of 7 days first at the nominal concentration of 0.4 µg/L, the growth rate µ even first at the nominal concentration of 0.8 µg/L. At the highest test nominal concentration 1.6 µg/l the growth of *Lemna gibba* was strongly inhibited. Based on all the results, the nominal test concentration 0.4 µg/L was determined as the 7-day LOEC.

Further, during the commenting period, new information regarding the validity criteria according current guidance (OCDE 221) and ECx values were required to the applicant and the following information was submitted:

Validity criteria:

The doubling time of frond number in the control must be less than 2.5 days (60 h), 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 this study was 2.3 days. Therefore, the validity criteria are met.

ECx calculations:

The following results were presented by the applicant: the nominal test item concentrations were 0.1, 0.2, 0.4, 0.8 and 1.6 µg a.s./L. The test concentrations were analytically verified and the correct preparation was demonstrated as the analytically determined concentrations correspond to 90 – 101% of the nominal test concentrations in the fresh test medium. At the end of the test period, the lowest test concentration was below the determination limit and not analysed. For the re-evaluation of the study, 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 are used, corresponding to 0.16, 0.34, 0.66 and 1.42 µg a.s./L.

Parameter	Growth rate (frond number) [µg a.s./L]	Yield (frond number) [µg a.s./L]	Growth rate (dry weight)[2] [µg a.s./L]	Yield (dry weight)[2] [µg a.s./L]
EC_{10}	0.28 (0.26 – 0.30)	0.31 (0.25 – 0.39)	0.62 (0.42 – 0.92)	0.38 (0.33 – 0.45)
EC_{20}	0.38 (0.36 – 0.40)	0.36 (0.30 – 0.45)	1.07 (0.81 – 1.42)	0.60 (0.54 – 0.67)
EC_{50}	0.71 (0.69 – 0.74)	0.49 (0.38 – 0.63)	> 1.42	1.41 (1.20 – 1.63)
NOEC	0.34	0.16	0.34 [1]	0.34 [1]

Values in parenthesis: lower and upper 95 % confidence level
[1] The NOEC was determined by visual determination of the raw data
[2] Initial dry weight of 5.95 mg (derived from number and dry weight of control fronds at the end and number of control fronds at start)

The NOEC for growth rate (frond number) is higher than the EC_{10}.

Taking into account the above information the results are:

7-d NOEC = 0.00034 mg SL-160/L (mean measured)

7-d E_rC_{50} = 0.0007 mg SL-160/L (mean measured)

7-d E_yC_{50} = 0.0005 mg SL-160/L (mean measured)

7-d E_rC_{10} = 0.00028 mg SL-160/L (mean measured)

The study is considered acceptable.

Author(s): Anonymous (1999).

Title: Toxicity of SL-160 to the aquatic higher plant *Lemna gibba* in a 21-d static growth inhibition test.

Guidelines: OPPTS 850.4400; OECD: Lemna growth inhibition test, dated June 1998.

GLP: Yes

The toxicity effects of Flazasulfuron on the growth of the aquatic plant *Lemna gibba* was determined in a 21-day static test. Exposure nominal concentrations were 0.04, 0.1, 1.0, and 5 µg/L. Aditionally, a control was tested in parallel.

Up to and including the nominal test concentration 0.1 µg/L the growth of *Lemna gibba* was not significantly lower than in the control. After 12 days the number of fronds in the control and the test concentrations of 0.04 and 0.1 µg/L was too high for the counting in the test flasks.

At the nominal test concentration 1.0 µg/L the number of fronds of *Lemna gibba* slowly increased during the test period, but the growth was much lower than in the control. The appearence of the test plants at this test concentration was strongly disturbed. At the highest nominal test concentration 5.0 µg/L the growth of *Lemna gibba* was nearly completely inhibited and the test plants were dead at the end of the test.

In conclusion, the test item had a clear inhibitory effect on the growth of *Lemna gibba* after the exposure period of 21 days at the nominal concentration of 1.0 µg/L. Thus, this test concentration was determined as the 21-day LOEC.

The 21-d NOEC was determined at the nominal concentration of 0.1 µg/L, since up to and including this test concentration no significant difference in growth was observed with respect to control up to at least the last quantification after 9 days, and no abnormalities and other signs of a toxic effect were observed at the test plants until the test end after 21 days.

The 7-day and 9-day EC50 were calculated for the growth parameters average specific growth rate µ and AUC. The 21-day EC50 could not be calculated since the growth in the controls could not be further quantified due to the high front densities.

Biological results

Parameter	**7-day EC_{50} in µg/L (95% confidence limits)**	**9-day EC_{50} in µg/L (95% confidence limits)**
Area Under growth curve (AUC)	0.5 (0.3-1.1)	0.5 (0.1-2.9)
Growth rate µ	1.1 (0.2-8.0)	1.1 (n.d)

The analytically determined concentrations of ^{14}C-SL-160 (P) in the fresh test media corresponded to 28.6, 45.3, 78.6 and 100 % in the days 21, 14, 7 and 0 of the experiment. It is considered that the endpoints should be expressed based on the measured concentrations.

Moreover, the author indicates that the 21-day EC_{50} could not be calculated due to the high front densities of the control.

This study was already considered during the previous evaluation process but not included in the LoEP (SANCO/3051/99/-Final). There are two more acute toxicity studies (see previous studies: Anonymous 1996 and Anonymous 1999) which evaluates the toxicity of SL-160 after 7 days exposure in *Lemna gibba*. Thus, this study is considered as supplementary information.

Author(s): Anonymous (2014).

Title: Toxicity of SL-160 TGAI to the aquatic plant *Myriophyllum aquaticum* in a static growth inhibition test with a prior rooting phase.

Guidelines: Ring Test Protocol: "Rooted Aquatic Macrophyte, Myriophyllum spec." (2009), Ring Test Protocol: "Standardized method for investigating test substance impact on rooted aquatic macrophyte".

Deviations: None

GLP: Yes

The toxicity of SL-160 on the growth of the aquatic macrophyte *Myriophyllum aquaticum* at nominal test concentrations of 0.0159, 0.05, 0.158, 0.5, 1.58 and 5.0 µg a.s./L was observed over a period of 14 days in a static test system. Three replicates per treatment and 6 replicates for the control, each replicate with three plants were used for the test. The day of application of the test item was designated as Day 0.

The pH-values were measured in the test media of all test concentrations and the control at test start, in the middle of the test and at test end.

At test start the shoot length above the sediment and the length of any side shoot was measured for all plants. At test end the shoot length and the length of any side shoot were measured. For the determination of the wet and dry weight five additional plants were prepared and kept simultaneously with the test beakers in the pre-culture period. From these five pots the plant biomass was determined to obtain the wet weight at test start. At test end the fresh and dry weight of every test plant was measured in the same manner as at the test start. Any sublethal symptoms e.g. chlorosis or necrosis were recorded once during the test (Day 4) and at the end of the test. At test end the existence of roots and their appearance were also recorded.

The concentrations of the test item were analysed in the duplicate test media samples from the nominal test concentrations 5.0, 1.58, and 0.5 µg test item/L from both sampling times. The lower nominal test concentrations 0.158, 0.05 and 0.0159 µg test item/L were not analysed, since they are far below the 7 day NOEC, determined in this test. LC-MS/MS was used for the determination of the test item.

Findings:

The pH values at test start 7.5 – 7.9, on day 4 7.4 – 7.5, at the end of the test 7.7 – 8.2.

At the start of the test 92 % of the nominal test concentration was found (average of the nominal test concentrations of 5.0, 1.58 and 0.5 µg test item/L). After 7 days test duration, 59 % of the nominal value was determined (average of the nominal test concentrations of 5.0, 1.58 and 0.5 µg test item/L). For the lowest test concentrations of 0.158, 0.05 and 0.0159 µg test item/L the geometric mean measured concentrations were estimated using the geometric mean measured recovery rate of the test concentration level of 0.5 µg test item/L. The concentrations found in the sediment and pore water are insignificant.

nominal test concentration [µg test item/L]	**fresh test media (0h)** % of nominal[1]	RSD [%]	n	**aged test media (7d)** % of nominal[2]	RSD [%]	n	**geometric mean[3]** concentration [mg test item/L]
control	n.a.	n.a.	2	n.a.	n.a.	2	-
0.0159	n.d.	-	-	n.d.	-	-	0.0114
0.05	n.d.	-	-	n.d.	-	-	0.0360
0.158	n.d.	-	-	n.d.	-	-	0.114
0.5	94	3	2	55	3	2	0.360
1.58	84	2	2	54	0	2	1.06
5	98	3	2	69	1	2	4.10

[1] mean value of all freshly prepared test media per treatment group
[2] mean value of all aged test media per treatment group
[3] geometric mean value per treatment group, for the lowest test concentrations of 0.158, 0.05 and 0.0159 µg test item/L the geometric mean measured concentrations were estimated using the geometric mean measured recovery rate of the test concentration level of 0.5 µg test item/L
RSD: relative standard deviation per treatment group
n: number of analysed samples
n.a.: not applicable
n.d.: not determined

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

No sub-lethal effects were observed. All plants developed roots. Effects on the roots occurred at the two highest test item concentrations. At nominal values of 1.58 and 5.0 µg test item/L the plants had less roots compared

to the control plants. Side shoots frequently occurred in the control and the lower concentration ranges but their numbers decreased with increasing test item concentrations.

Influence of SL-160 on the growth of *Myriophyllum aquaticum.*

Parameter	Yield (shoot length)	Growth rate (shoot length)	Yield (wet weight)	Growth rate (wet weight)	Yield (dry weight)	Growth rate (dry weight)
	[µg a.s./L]					
EC_{50} (7-day)	2.70	> 4.10	3.15	3.67	> 4.10	> 4.10
95 % conf. limits	1.79 - > 4.10	3.75 - > 4.10	1.11 - > 4.10	n.d.	n.d.	n.d.
EC_{20} (7-day)	1.08	1.74	0.77	1.05	> 4.10	> 4.10
95 % conf. limits	0.37 – 1.66	0.95 – 2.33	< 0.01 – 2.10	n.d.	n.d.	n.d.
EC_{10} (7-day)	0.67	1.02	0.37	0.55	> 4.10	> 4.10
95 % conf. limits	0.14 - 1.15	0.37 – 1.54	< 0.01 – 1.06	n.d.	n.d.	n.d.
7-day NOEC	1.06	1.06	1.06	1.06	4.10	4.10
7-day LOEC	4.10	4.10	4.10	4.10	> 4.10	> 4.10

nd: not determinable
Values refer to geometric mean test concentrations

RMS comments: the design of test included three replicates (not four as recommends the OECD test 239). It is noted that when this study was conducted not definitive OECD guideline 239 was available.

For the test item concentration at which NOEC was derived, the recovery at day 7 was 54% of nominal concentration.

The analysis of flazasulfuron in test media during the experiment was performed only in the three highest test item concentrations.

The design of the test includes 7 treatment groups (1 Control and 6 treatments) with three replicates. However, it is noted that the endpoints were obtained based on the geometric mean measured concentrations on day 0 and day 7 of each test item concentration using only two replicates. Moreover, the validity criteria were not completely fulfilled.

It is noted that the proposed 7-day NOECs are higher than 7-days ECs_{10}.

Author(s): Anonymous (2014).

Title: Toxicity of SL-160 TGAI to the Reed Mannagras *Glyceria maxima* in a Static Growth Inhibition Test.

Guidelines: OECD 239.

Deviations: None

GLP: Yes

The toxicity of SL-160 on the growth of the aquatic reed mannagras Glyceria maxima at nominal test concentrations of 1.00, 3.17, 10.0, 31.6 and 100 µg a.s/L was observed over a period of 28 days in a static test system.

The pH-values were measured in the test media of all test concentrations and the control at test start, in the middle of the test and at test end.

At test start the shoot length above the sediment was measured for all plants. Shoot length above the sediment was measured at test start, at Day 7, 14 and 21 and at test end. Plant shoots of additional plants are used at test start to determine wet and dry weight. At test end fresh and dry weight of every test plant was measured by cutting the plant at the sediment surface. Any sublethal symptoms e.g. chlorosis or necrosis were recorded three times during the test (Day 7, 14 and 21) and at the end of the test. At test end the existence of roots and their appearance were also recorded.

One sample from the freshly prepared stock solution and duplicate samples from the freshly prepared test media of all test concentrations and the controls and sediment samples from the highest nominal test concentration 100 µg test item/L and from the controls were taken at the start of the test. At the end of the test duplicate samples were taken from all test media and the control by pouring together the contents of each treatment. Sediment samples from the highest nominal test concentration 100 µg test item/L and from the controls were taken at the end of the test. LC-MS/MS was used for the determination of the test item.

Findings:

The pH value at different test days:
- test start: 7.8 – 8.0,
- on day 7: 8.1 - 8.2,
- on day 14: 8.4 - 8.8,
- on day 21: 8.8 - 9.6,
- at the end of the test: 8.5 - 9.3.

At the start of the test 119 % of the nominal test concentration was found in the test media (average of all nominal test concentrations). After 28 days test duration, 19 % of the nominal value was determined in the test media (average of all nominal test concentrations). The concentrations found in the sediment and pore water are insignificant.

nominal test concentration [µg test item/L]	fresh test media (0h) % of nominal[1]	RSD [%]	n	aged test media (28d) % of nominal[2]	RSD [%]	n	geometric mean[3] concentration [µg test item/L]
control	n.a.	n.a.	2	n.a.	n.a.	2	n.a.
1	127	12	2	25	31	2	0.563
3.17	124	7	2	19	0	2	1.54
10	114	2	2	17	4	2	4.40
31.6	111	4	2	15	0	2	12.9
100	120	3	2	19	11	2	47.7

[1] mean value of all freshly prepared test media per treatment group
[2] mean value of all aged test media per treatment group
[3] geometric mean value per treatment group calculated using mean recovery rates of freshly prepared and aged test media, rounded to 3 significant digits
RSD: relative standard deviation per treatment group
n: number of analysed samples; n.a.: not applicable

All chemical and physical parameters (dissolved oxygen concentration, pH and temperature) in the definitive test were within expected ranges.

No sub-lethal effects were recorded for all test concentrations. Rhizomes frequently occurred in all test item concentrations.

Influence of SL-160 on the growth of *Glyceria maxima.*

Parameter	Yield (shoot length)	Growth rate (shoot length)	Yield (wet weight)	Growth rate (wet weight)	Yield (dry weight)	Growth rate (dry weight)
	[µg a.s./L]					
EC_{50} (28-day)	22.5	24.2	> 47.7	> 47.7	> 47.7	> 47.7
95 % conf. limits	15.9 – 34.5	17.9 – 34.7	n.d.	n.d.	n.d.	n.d.
EC_{20} (28-day)	6.83	7.41	26.4	36.4	> 47.7	> 47.7
95 % conf. limits	3.23 - 10.3	4.03 - 10.6	10.1 - 41.8	18.2 - 71.2	n.d.	n.d.
EC_{10} (28-day)	3.66	3.99	12.7	17.0	> 47.7	> 47.7
95 % conf. limits	1.23 - 6.24	1.64 - 6.44	1.11 - 21.8	1.18 - 27.8	n.d.	n.d.
28-day NOEC	4.40	4.40	12.9	12.9	47.7	47.7
28-day LOEC	12.9	12.9	47.7	47.7	> 47.7	> 47.7

nd: not determinable
Values refer to geometric mean measured test concentrations

Rapporteur Member State (RMS) commented:

The concentrations of the test item SL-160 were analysed after 28 days in the test media samples from all test concentrations and control samples. Since the decrease of the test item concentration, the biological results are related to geometric mean measured test concentration.

A decrease in growth rate based on wet weight was only statistically significant in the 100 µg/L (measured concentration 47.7 µg a.s./L) treatment group.

Although not statistically significant, the reduction in frond at the 31.6 mg/L (measured concentration 12.9 µg/l) test concentration can be considered biologically meaningful.

Nominal concentration	Yields y (wet weight) and % inhibition of y (after 28 days)			Growth rates µ (wet weight) and % inhibition of µ (after 28 days)		
[µg test item/L]	y [mg]	%		µ [1/day]	%	
Control	684.9	-		0.020	-	
1.00	723.5	-5.6		0.021	-5.8	
3.17	1074.9	-56.9		0.028	-38.2	
10.0	950.3	-38.7		0.026	-29.1	
31.6	679.8	0.7		0.020	0.0	
100	474.0	30.8	*	0.015	24.4	*

- % inhibition: increase in growth relative to that of control
* mean value significantly different from the control (determined directly from raw data)

Consequently, RMS recommends decreasing the 7-days growth rate NOEC (based on wet weight) to 10 µg/l corresponding to 4.4 µg a.s./L

During the commenting period, new information regarding the fulfilment of the new relevant validity criteria were required to the applicant. The following information was submitted:

In the Glyceria maxima growth inhibition study with SL-160 technical, data on shoot length, wet weight and dry weight were recorded. The shoot length, wet weight and dry weight control data are shown in table below, together with the respective growth rates as reported in the study.

Replicate		Shoot length [cm]		Wet weight [mg]	Dry weight [mg]
	Plant No.	0 days	28 days		
Aquarium 1	1	48.9	70	1432.8	489.8
	2	34.9	42.3	1313.8	537.8
	3	43.2	56.9	1719.8	639.8
	4	51.2	51.8	1415.8	779.8
	5	20.9	38.4	747.8	212.8
Aquarium 2	1	50.9	80.3	1587.8	487.8
	2	48.5	54.2	1485.8	403.8
	3	59.3	96.6	1688.8	509.8
	4	53.4	71.3	2328.8	819.8
	5	43.1	64.7	2103.8	765.8
Aquarium 3	1	40.4	59.2	1631.8	561.8
	2	52.2	70.6	1691.8	600.8
	3	36.5	54.2	1373.8	533.8
	4	53.1	59.4	1887.8	648.8
	5	28.0	44.8	1013.8	354.8
Growth rate [µ]		0.011		0.015	0.044
Coefficient of variation [%]		23.8	25.0	25.1	29.4
Doubling time [d]		63.0		46.2	15.6

In addition, the coefficient of variation in the control cultures were calculated for shoot length, wet weight and dry weight and the doubling times for the three parameters were calculated by the following formula:

- *$Td = ln(2)/\mu$*
- *With Td = doubling time*
- *μ = growth rate*

The coefficients of variation for the parameters shoot length, wet weight and dry weight are between 23.8 and 29.4 % in the control cultures between the replicates. Thus, the coefficients of variation are lower than 35 %, the trigger for validity which is given e.g. for the algal growth inhibition study (OECD 201) and sediment-free Myriophyllum spicatum toxicity test (OECD 238). The doubling time is between 15.6 and 63.0 d for the three parameters. However, this value cannot be compared with triggers given in available OECD guidelines, as this parameter is highly variable for different test species (slow and fast growing species).

RMS agrees with the applicant statement.

Endpoints accepted:

28-days NOEC = 0.00440 mg/L (measured concentration)

28-days ErC_{50} = 0.0242 mg/L (measured concentration)

28-days E_yC_{50} = 0.0225 mg/L (measured concentration)

28-days ErC_{10} = 0.00399 mg/L (measured concentration)

<u>METABOLITES.</u>

The available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA, ADMP, HTPP) revealed toxicity values > 1 mg/L and they present low level of toxicity. Therefore, the studies with these metabolites are not described here in detail but they are included in Annex I to this dossier. In any case, these studies were undertaken according to OECD 221 and to GLP and are considered reliable.

Apart from these studies, there is an acute toxicity test for metabolite SSRE-018 (GTPS) which revealed toxicity values less than 1 mg/L and it is summarised below.

Author(s): Anonymous (2014).

Title: Toxicity of SSRE-018 to the aquatic plant *Lemna gibba* in a semi-static growth inhibition test.

Guidelines: OECD 221.

Deviations: None

GLP: Yes

The toxicity of SSRE-018 on the growth of duckweed (*Lemna gibba*) at nominal test concentrations of 3.2, 10, 32, 100, 320 and 1000 µg a.s./L was observed over a period of 7 days in a semi-static test system. The frond numbers were determined on day 3, 5 and 7. The dry weight of each replicate at test end was determined.

The concentrations of the test item SSRE-018 were also analysed after 3, 5 and 7 days in the test media samples from all test concentrations and control samples. The quantification of the test item was performed using liquid chromatography (HPLC-UV).

ECx values could not be calculated due to absence of effects. The LOEC and NOEC values for yield and growth were calculated by Williams t-test.

Findings:

The pH values: 7.6-7.9 (test start) and 8.6-9.1 (test end).

The doubling time of frond number in the control was found to be 1.6 days and was therefore less than 2.5 days, as required by the guideline.

In the freshly prepared test media, 110% of the nominal test concentration was found (average of all test concentrations). In the aged test media, 23% of the nominal value was determined (average of all test concentrations, except for 3.2 and 10 µg a.s./L, were some values had been below LOQ). Geometric mean values of the test concentration level of 3.2 and partially of 10 µg/L were calculated with half the LOQ (1.25 µg test item/L) since the values found were below the LOQ. Thus, as the test item was not stable during the renewal periods, all reported results refer to geometric mean concentrations.

Conclusions:

All reported 7 d EC_{50}, EC_{20} and EC_{10} values for growth rate (based on frond number and dry weight) and yield (based on frond number and dry weight) have been reported to be > 460 µg a.s./L, the highest tested concentration. Respectively, all NOEC values for yield (based on frond number and dry weight) and for growth rate (based on frond number and dry weight) are reported to be > 460 µg a.s./L. All corresponding LOEC values are reported to be > 460 µg a.s/L.

7-day NOEC > 0.460 mg SSRE-018/L

7-day E_rC_{50} > 0.460 mg SSRE-018/L

7-day E_yC_{50} > 0.460 mg SSRE-018/L.

7-day E_rC_{10} > 0.460 mg SSRE-018/L

The study is acceptable.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data are available.

11.6 Long-term aquatic hazard

A brief summary of the aquatic toxicity studies evaluated during Annex I inclusion of Flazasulfuron and submitted for the purposes of EU renewal is reported below. From all available ecotoxicity tests on this substance only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 113: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Fish early life-stage. OECD 210; OPPTS 850.1400	Rainbow trout (*Oncorhynchus mykiss*)	Flazasulfuron technical (96.7% purity)	89d-NOEC = 20 mg/L, based on nominal concentrations.	The study is considered acceptable.	Anonymous (2004)
Long term and chronic toxicity to aquatic invertebrates. OECD 211; OPPTS 850.1300; ASTM E 1193-97	*Daphnia magna*	Flazasulfuron technical (98.5% purity)	21-day NOEC (immobility) = 6.2 mg/L, based on mean measured concentrations.	The study is considered acceptable.	Anonymous (2013)
Long term and chronic toxicity to aquatic invertebrates. OECD 202 (April 1984). Proposed updated version OECD 202 (June 1993).	*Daphnia magna*	Flazasulfuron technical (95.1% purity)	21-day NOEC = 6.25 mg/L, based on nominal concentrations	The study is considered acceptable.	Anonymous (1995).
Acute toxicity to algae or other aquatic plants. OECD Guideline 201	Green alga (*Pseudokirchneriella subcapitata)*	Flazasulfruon technical	72h-NOE_rC = 0.018 mg/L 72h-E_rC_{10} = 0.030 mg/L based on geometric mean concentration.	The validity criteria were not met. Supplementary information.	Anonymous (1995).
Acute toxicity to algae or other aquatic plants. OECD Guideline 201	Freshwater green alga (*Pseudokirchneriella subcapitata*)	Flazasulfuron technical (98.96 % purity) The pH ranged from 7.9 to 9.3	72h-NOE_rC = 0.0010 mg/L, 72h-E_rC_{10} = 0.0018 mg/L	The study is considered acceptable.	Anonymous. (2011).

Method	Species	Test material	Results[1]	Remarks	Reference
			based on mean measured concentrations		
Acute toxicity to algae or other aquatic plants. OECD 201; Directive 92/69/EEC, C3 (1992).	*Anabaena flos-aquae.*	Flazasulfuron technical (97.3% purity) The pH ranged from 7.7 to 10.2.	72h-NOE_rC = 0.017 mg/L 72h-E_rC_{10} = 0.0368 mg/L based on geometric mean concentration.	No fulfilment of the validity criteria and the impossibility to calculate an EC_{50}. Supplementary information.	Anonymous (1996)
Acute toxicity to algae or other aquatic plants. US EPA 797.1160	Duckweed (*Lemna gibba)*	Flazasulfuron technical (97.3% purity) The pH ranged from 5.0 to 5.3.	7d-NOE_rC = 0.00002 mg/L 7d-E_rC_{10} = 0.000027 mg/L .	There are some uncertainties related to analytical results and the validity criteria were not met Supplementary information.	Anonymous (1996)
Acute toxicity to algae or other aquatic plants. OPPTS 850.4400; OECD 221	Duckweed (*Lemna gibba*)	Flazasulfuron technical (99.7% purity) The pH ranged from 7.4 to 8.9.	7d-NOE_rC (frond number) = 0.00034 mg/L **7d-E_rC_{10} (frond number) = 0.00028 mg/L (mm)** based on mean measured concentrations	The study is considered acceptable.	Anonymous (1999)
Acute toxicity to algae or other aquatic plants.	Duckweed (*Lemna gibba)*	Flazasulfuron technical	21d-NOEC = 0.0001 mg/L	This study was considered in the previous evaluation process but not included in the LoEP.	Anonymous (1999).

Method	Species	Test material	Results[1]	Remarks	Reference
OPPTS 850.4400; OECD 221				Supplementary information.	
Acute toxicity to algae or other aquatic plants. Ring Test Protocol: "Rooted Aquatic Macrophyte, Myriophyllum spec." (2009), Ring Test Protocol: "Standardized method for investigating test substance impact on rooted aquatic macrophyte	*Myriophyllum aquaticum*	Flazasulfuron technical (98.5% purity) The pH ranged from 7.4 to 8.2.	7d-NOE_rC (shoot length) = 0.00106 mg/L 7d-E_rC_{10}(shoot length) = 0.00102 mg/L 7d-E_rC_{20}(shoot length) = 0.00174 mg/L	The validity criteria were not completely fulfilled. Supplementary information.	Anonymous (2014).
Acute toxicity to algae or other aquatic plants. OECD 239.	*Glyceria maxima*	Flazasulfuron technical (98.5% purity). The pH ranged from 7.8 to 9.6.	28d-NOE_rC = 0.0044 mg/L 28d-ErC_{10} = 0.00399 mg/L based on mean measured concentration	The study is considered acceptable.	Anonymous (2014)
Acute toxicity to algae or other aquatic plants. OECD 221	Duckweed (*Lemna gibba*)	SSRE-018 (97.0% purity) The pH ranged from 7.6 to 9.1.	7d-NOEC > 0.460 mg/L 7d-E_rC_{10} > 0.460 mg/L	The study is considered acceptable.	Anonymous (2014).
Chronic toxicity to sediment dwelling organisms	Midge larvae (*Chironomus riparius*)	Flazasulfuron technical (99.7% purity)	23-dy NOEC = 0.1 mg/L, based on nominal concentration.	The study is considered acceptable.	Anonymous (2000).

Method	Species	Test material	Results[1]	Remarks	Reference
OECD draft guideline, May 1998; BBA Guideline proposal (1995)		The pH ranged from 6.6 to 7.8.			

11.6.1 Chronic toxicity to fish

Fish early life stage toxicity tests.

Author(s): Anonymous (2004).

Title: SL-160 technical: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*).

Guidelines: OECD 210: OPPTS 850.1400

GLP: Yes

The purpose of this study was to investigate the effects of SL-160 technical (Flazasulfuron) on the survival and developments of early life-stage Rainbow Trout (*Oncorhynchus mykiss)*. The exposure period was 89 days (60 days post-hatch) under flow-through conditions. Exposure nominal concentrations were 1.3, 3.2, 8.0, 20 and 50 mg/L of Flazasulfuron, corresponding to measured concentration of 1.3, 3.0, 7.0, 17 and 42 mg/L of Flazasulfuron. The treatment groups and control consisted of two replicate test aquaria. The exposure was initiated when the egg incubation cups, each containing 100 embryos, were distributed to each of the 12 test aquaria. Observations on hatch, development, survival and abnormal appearance of behaviour were made daily. Survival rates, body weights and lengths were determined at the end of exposure.

Findings:

The analytical results established that the expected exposure concentration-gradient was maintained during the 89-day test. The mean measured concentrations ranged from 83 to 99% of nominal concentrations and defined the treatment levels tested as 1.3, 3.0, 7.0, 17 and 42 mg a.s./L.

Mean measured concentrations of SL-160 established during the early life-stage exposure of *Oncorhynchus mykiss*

Nominal concentration (mg/L)	Mean measured concentration [mg a.s./L]	% of nominal
1.3	1.3 ± 0.092	99
3.2	3.0 ± 0.23	95
8.0	7.0 ± 0.54	87
20	17 ± 1.3	86
50	42 ± 3.6	83

Validity criteria.

According to the validity criteria of OCDE 210 *"the water temperature should not differ by more than + 1.5 ºC between test chambers or between successive days at any time during the test, and should be within the*

temperature ranges specified for the test species". According to the study summary the temperature was between 10-14 ºC. After EFSA requirement, the following statement was submitted by the applicant: *The temperature deviations of the study have been noted. However hatching and post-hatch success of 99% and 95% demonstrate a successful study and data of the study satisfy the data requirement.* RMS agrees with the applicant statement.

Moreover, during the commenting period the applicant was required to provide ECx and NOEC values for long-term studies. The following information was submitted by the applicant:

The endpoints hatchability, post-hatch survival, fresh weight, dry weight and length were analysed. The results of the re-evaluation of the study using ToxRat (v. 3.2.1) are presented below.

	NOEC	LOEC	LC_{10}/EC_{10}*	LC_{20}/EC_{20}*	LC_{50}/EC_{50}*	Statistical Method
	[mg a.s./L]					
Hatchability on day 39	20	50	23.76	30.61	47.8	Logit
Post-hatch survival (60 days post hatch)	20	50	not reliable	not reliable	not reliable	Probit
Fresh weight (60 days post hatch)	20	> 20	n.d.	n.d.	n.d.	Probit
Dry weight (60 days post hatch)	20	> 20	n.d.	n.d.	n.d.	Probit
Length (60 days post hatch)	20	> 20	n.d.	n.d.	n.d.	Probit

n.d. - not determined due to statistical reasons
* For hatchability and post-hatch survival the endpoints are reported as LC_{10} and LC_{20}. For fresh weight and length the endpoints are reported as EC_{10} and EC_{20}.

<u>*Hatchability:*</u>

Although EC_{10}, EC_{20} and EC_{50} could be calculated for hatchability, due to a tight dose-response relationship between the two highest concentrations, i.e. the NOEC and the LOEC, the 95% confidence interval cannot be determined. However, the calculated EC_{10} and EC_{20} fall between the established NOEC and LOEC and are therefore considered valid.

<u>*Post-hatch survival:*</u>

In reference to EFSA Technical Report 'Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology' (2014), studies conducted prior to changes in data requirements (i.e. provision of EC_{10} and EC_{20}) have been rated for reliability of ECX. In such cases where an EC_{10} and EC_{20} cannot be reliably calculated, the NOEC should be 'retained as primary endpoint'. The statistical re-evaluation for post-hatch survival indicates a clear lack of dose response. Thus the calculation of ECX values is considered to be not reliable.

Fresh weights, dry weights, and length:

Due to the lacking concentration/response the EC/LCx could not be determined.

The RMS agrees with the endpoint proposed:

89-d NOEC = 20 mg/L.

11.6.2 Chronic toxicity to aquatic invertebrates

Author(s): Anonymous (2013).

Title: Flazasulfuron: a semi-static life-cycle toxicity test with the Cladoceran (*Daphnia magna*).

Guidelines: OECD 211; OPPTS 850.1300; ASTM E 1193-97.

GLP: Yes

The purpose of this study was to investigate the effects of SL-160 (Flazasulfuron) on the reproduction of *Daphnia magna* over an exposure period of 21 days, under semi-static conditions. The test was conducted at nominal test concentrations of 3.1, 6.3, 13, 25 and 50 mg a.s./L corresponding to measured concentrations of 3.0, 6.2, 13, 25, and 48 mg a.s./L, with the addition of a control (without the test item). Ten replicate test chambers containing one daphnid each were tested for each treatment group and 20 replicate test chambers were tested for the control group. The assessments of adult worm survival, reproduction, and growth were carried out after 21 days of exposure.

Test solutions were renewed every 2-3 days. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at test initiation, at the beginning and end of the longest renewal cycle each week, and at test termination.

First-generation daphnids were observed daily during the test for mortality, the onset of reproduction, and clinical signs of toxicity. Following the onset of reproduction, the numbers of second-generation daphnids were counted three times per week, and at test termination (day 21). Body lengths and dry weights of the surviving first-generation daphnids were measured at the end of the exposure period.

Test endpoints analysed statistically for first-generation daphnids were survival, reproduction (the number of live young produced per surviving first-generation daphnid), and growth (length and dry weight).

Findings:
In this study, the mean measured test concentrations were determined from samples of test water collected from each treatment and control group along all-time experiment which represented from 96% to 100% of nominal concentration.

RMS considers immobility as the more suitable effects to quantify an endpoint in this study. The rest of effects (reproduction and growth) were not treatment related and consequently, the values of EC_{50} and EC_{10} should be considered inaccurate.

The author considers relevant the decrease of survival observed at treatment 25 mg as/L although it is not statistically significant. RMS, noted there is a clear dose-response relationship, considering this effect at 13

mg as/L. Therefore, RMS recommends decrease the value of 21d-NOEC immobility (survival) from 13 mg as/L to 6.2 mg as/L.

The study is considered acceptable.

Author(s): Anonymous (1995).

Title: Influence of SL-160 technical on reproduction of *Daphnia magna*.

Guidelines: OECD Guideline for Testing OF Chemicals 202, Part II: Reproduction Test at least 14 Days, Paris/France, adopted April 1984; Proposed updated version of OECD Guideline 202, Part II (June 1993).

GLP: Yes

The purpose of this study was to investigate the influence of SL-160 (Flazasulfuron) on the reproduction and survival rate of *Daphnia magna* over an exposure period of 21 days, under semi-static conditions. The range of nominal concentrations was from 0.39 mg/L to 100 mg/L which were spaced by a geometric progression factor of 4. Thus, the 0.39 mg/L, 1.56 mg/L, 6.25 mg/L, 25 mg/L and 100 mg/L as well as the control were analysed by HPLC at the beginning and at the end of the first (day 0 and 2) and the last (day 19 and 21) exposure periods. The test was prolonged until day 23 because control and SL-160 technical-treated animals have started to release offspring.

The mortality of adult animals and the number of young produced by the adults were checked three times per week. Between day 19 and 21, checks were performed daily and on day 23 because the validity criterion of the reproduction of at least 60 young daphnids. Dead animals and eggs on the bottom of the test vessels were removed. The offspring were removed too and counted.

For the validity of the test, the total mean offspring in the control should be at least 60 per parent animal. The mortality in the control and at the end of the test < 10%. The coefficient of variation around the mean of the number of living offspring produced per parent animal in the control should be ≤ 25%. The dissolved oxygen concentration should remain >3 mg/l.

On each day of the test medium renewal, the pH and the oxygen of the control, of the lowest and highest test concentration was measured.

Findings:

The analytical results were:

Time (days)	Mean measured concentration (mg/L)	%
0.39 mg/L		
0	0.3905	100.1
2	0.3651	93.6
19	0.3095	79.4
21	0.3013	77.3
23	0.3925	100.6
6.25 mg/L		

0	6.317	101.1
2	5.999	96.0
19	6.416	102.7
21	6.216	99.5
23	7.005	112.1
25 mg/L		
19	25.13	100.5
21	24.04	96.2
23	30.08	120.3
100 mg/L		
0	103.1	103.1
2	96.23	96.23

After 23-day exposure period, no immobility was observed in the control group as well as the test article concentration 0.39, 1.56 and 6.25 mg/L. The 50% immobility, was observed at 25.0 mg/L. The 100% immobility, was observed at 100 mg/L after 12 days of exposure.

The EC50 of the immobility of the parental generation at the end of the test was calculated by the Logit model to be at 25.0 mg/L with the 95% confidence limits of 15.3-40.9 mg/L.

Reproduction of young daphnids started between day 7 and 9 in the control group and between day 9 and 12 in the treated groups.

The influence of the test article on the reproductive output was statistically not significant in comparison to the control group from 0.39 mg/L to 6.25 mg/L test concentration. At 25 mg/L the influence was statically significant in comparison with the control group. No statistical test could be applied at the 100 mg/L test concentration due to 100% immobility of the parent animals within 12 days of exposure.

The 21d-NOEC for the reproductive capacity was found to be at 6.25 mg/L, and the LOEC was found to be at 25 mg/L (nominal concentration).

The study is considered acceptable.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to previous point 11.5.3 where the toxicity tests with the parent on algae and Lemna are included.

11.6.4 Chronic toxicity to other aquatic organisms

Sediment dwelling organisms

Author(s): Anonymous (2000).

Title: Effects of SL-160 on the development of sediment larvae of *Chironomus riparius* in a water-sediment system.

Guidelines: BBA proposal guideline (1195); OECD draft guideline (1998).

GLP: Yes

First-instar larvae of Chrironomus riparius were exposed for a period of 23 days, sufficient to assess the impact on full maturation of the larvae to adult midges.

The radiolabeled test item (14C-SL-160 (P)) was applied to the water column in a static sediment water system to simulate spray drift or overspray. A limit concentration of initial nominal 100 µg/L of SL-160 was tested. A control group was tested in parallel.

Findings:

During the test period the pH-values in the test media ranged from ph 6.6 to 7.8. The dissolved oxygen concentrations were with at least 5.9 mg/l.

The analytically measured concentration of Flazasulfuron in the test medium one hour after application corresponded well with the nominal concentrations. Therefore, all reported biological results are related to the nominal initial concentration of Flazasulfuron in the water column. The concentration of Flazasulfuron in the water phase had decreased from 97 µg/L at test start to approximately 59 and 48 µg/L at day 7 and 23, respectively. Also in pore water the concentration of the test substance had decreased from day 7 (22 µg/L) to day 23 (<01 µg/L). However, in the sediment the concentration of Flazasulfuron had increased to about 66 µg/kg (8.2% AR) dry sediment at day 23.

Seven different metabolites (radioactive fractions) of the parent molecule were detected in the water phase, pore water or sediments. The main metabolite was DTPU with approximately 21% and 24% of initially radioactivity on day 7 and 23, respectively (total amount in whole system).

The total radioactive residue in the overlaying water column had decreased nearly constantly from 97 µg/L after application to about 78 µg/L after 7 days, and to 65 µg/L at the test termination after 23 days. In the pore water concentration of radioactive residues was with about 37 and 35 µg/L after 7 and 23 days nearly constant.

Different to the pore water concentrations, the radioactivity in the sediments had continuously increased during the test period to about 215 µg/kg dry sediment at day 23. The mayor part of radioactivity in sediments was extractable and not strongly bound to sediment.

At the concentration of initial 100 µg/L of Flazasulfuron the mean emergence rate and the mean development rate of the midges were not significantly lower than in the control. Also no signs of intoxication were observed at the larvae, pupae and emerged midges during the test in the treated and untreated systems.

Based on nominal concentration:

23-day NOEC = 0.1 mg Flazasulfuron/L.

<u>METABOLITES</u>

There are available acute toxicity tests for metabolites of Flazasulfuron DTPU and HTPP on *Chironomus riparius*, which revealed toxicity values > 1 mg/L (LC_{50} > 100 mg/L). Therefore, these studies are not described here in detail but they are included in Annex I to this dossier.

11.7 Comparison with the CLP criteria

Endpoint	CLP classification criteria	Flazasulfuron data	Conclusions
Rapid degradability	Demonstrated rapid/not rapid degradation	Not readily biodegradable and not rapidly degradable	Not rapidly degradable
Short-term toxicity	LC_{50}/EC_{50} value	Adequate data for fish, aquatic invertebrates, algae and aquatic plants. 7d-E_rC_{50} = 0.0007 mg/L, Duckweed (*Lemna gibba*) (Anonymous, 1999).	Aquatic Acute 1
Long-term toxicity	NOEC or EC10 value	Adequate data for fish, aquatic invertebrates, algae and aquatic plants. 7-day ErC_{10} = 0.00028 mg/L Duckweed (*Lemna gibba*) (Anonymous, 1999)	Aquatic Chronic 1
Bioaccumulation potential	log Kow ≥ 4	log K_{ow} = 1.30 at pH 5 at 25 °C log K_{ow} < -0.06 at pH 7 at 25 °C	Bioconcentration is not expected

11.7.1 Acute aquatic hazard

Full acute data set was available for Flazasulfuron as there were acute studies on fish, aquatic invertebrates, algae and aquatic plants, covering the three trophic levels. Also studies with metabolites (DTPU, DTPP, TPSA and ADMP) were available for all trophic levels although only GTPS presented a toxicity value < 1 mg/L on aquatic macrophyte (*Lemna gibba*). However, classification proposal is based on studies conducted with Flazasulfuron as the lowest and the most reliable endpoint values.

Based on the available data, the lowest acute toxicity endpoint is *Lemna gibba* **E_rC_{50}(7d)= 0.0007 mg/L** (Anonymous 1999). This endpoint will establish the M factor needed for the CLP environmental classification.

It is concluded that Flazasulfuron does fulfil the criteria for classification and it should be classified according to Regulation (EC) No. 1272/2008 as:

Aquatic Acute 1 with M factor of 1000.

CLP criteria:

- for EC_{50} acute toxicity values below or equal to 1 mg/l [E_rC_{50}(7d) = 0.0007 mg/L ≤ 1 mg/L] and
- for M factor, acute toxicity value in the range 0.0001 < L(E)C50 ≤ 0.001 mg/L.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation

The log Kow values for Flazasulfuron is 1.3 at pH 5 and less than 0.06 for pH 7, which are less than the CLP log Kow trigger value of > 4 intended to identify substances with a potential to bioaccumulate under CLP criteria. According to CLP guidance, measured estimates should be used in preference when available to conclude on the bioaccumulation potential of a substance (BCF ≥ 500 indicates bioaccumulation potential). Since, no studies are available to establish measured BCF estimates, log Kow data have been used to conclude on the potential for bioaccumulation of Flazasulfuron. Both log Kow estimates are lower than the CLP trigger value of 4 and, therefore, Flazasulfuron is considered to have low potential to bioaccumulate.

Degradation

A ready biodegradability test (OECD test guideline 301B) shows Flazasulfuron being not readily biodegradable for purposes of classification as the pass level criteria of ready biodegradation test (70% of DOC removal or 60% of theoretical oxygen demand) within 28 days was not reached (0% biodegradation in 28 days).

Flazasulfuron in not hydrolytically stable at pH values of 4, 5, 7 and 9 at 22ºC and 37ºC under sterile conditions in the dark for 30 days, and degradation products have been detected (DTPU, DTPP, TPSA and ADMP). At pH 5 Flazasulfuron degradates rapidly with half live between 3.44 and 3.68 days and at pH 7 the half live value are between 15.7 and 16 days.

In an aerobic mineralization study Flazasulfuron degraded with DT_{50} values of 21 and 25, depending on test concentration, to the following metabolites: DTPU and DTPP. Mineralisation reaches a maximum of 0.6% after 90 days.

The whole system half live in a natural water/sediment study is 23.9 days and formation of metabolites is the main route of degradation of Flazasulfuron in water/sediment systems. At the end of this study, the maximum carbon dioxide was 0.65% of the applied dose (pH 6.6 system) indicating minimal mineralization. Flazasulfuron can be considered as not rapidly degradable in the aquatic environment from the water/sediment system study carried out.

Regarding photodegradation, degradation of Flazasulfuron was observed in both the treated irradiated samples (DT_{50} 16.5d) and dark control samples (DT_{50} 5.7d) indicating that hydrolysis is the mechanism of reaction. Photolysis is not expected to be a major route of degradation.

Overall, degradation information does not provide sufficient data to show that Flazasulfuron is ultimately degraded to > 70% within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, Flazasulfuron is considered being **not rapidly degradable** according to the CLP criteria.

Toxicity

Long-term aquatic toxicity data regarding technical Flazasulfuron are available for fish, aquatic invertebrates including sediment dwelling organisms, algae and other aquatic plants (i.e. there is appropriate data for all three trophic levels that need to be assessed for CLP classification). Classification proposal is based on studies conducted with Flazasulfuron although there were acute and chronic studies available for metabolites. However, the available acute toxicity data for relevant metabolites of Flazasulfuron (DTPU, DTPP, TPSA,

ADMP, HTPP) revealed toxicity values > 1 mg/L and they present low level of toxicity. Apart from these data, there is an acute toxicity test for metabolite SSRE-018 (GTPS) which revealed toxicity values less than 1 mg/L 7-day E_rC_{10} (*Lemna gibba*) > 0.460 mg SSRE-018/L but these values are much higher than toxicity values for active substance.

The lowest NOErC value is the measured **7d-ErC_{10} of 0.00028 mg a.s./L** for duckweed *(Lemna gibba)* (Anonymous, 1999). This is > 0.0001 mg/L but ≤ 0.001 mg/L, and since Flazasulfuron is considered to be 'not rapidly degradable' as well as not potentially bioaccumulative, it should be classified according to Regulation (EC) No. 1272/2008 as:

Aquatic Chronic 1 with a chronic M-factor of 100.

CLP Criteria:

- Aquatic long-term toxicity reflected by a valid endpoint for aquatic macrophytes reproduction ErC_{10} (7d)=0.00028 mg/L, and
- For the M factor, Flazasulfuron is considered not rapidly degradable substance and its long-term toxicity value is in the range of 0.0001 to 0.001 (ErC_{10} = 0.00028 mg/L).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Taking into account all the information and the assessment summarized in the previous sections 11.7.1 and 11.7.2, the following classification class and category can be concluded for this active substance:

According to Table 4.1.0 (a) and (b)(i), Flazasulfuron meets the CLP Regulation criteria for being classified as **Aquatic Acute 1 with M factor of 1000** and **Aquatic Chronic 1 with M factor of 100.**

Therefore, the proposal for classification for Flazasulfuron is:

Aquatic Acute 1; H400: Very toxic to aquatic life. M-factor 1000

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects. M-factor 100

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Global effects such as contributions to global warming potential (GWP), ozone depleting potential (OPD) and photochemical ozone creation potential (POCP) are considered if there is a high probability for evaporation and persistence in the gas phase, which can be expressed by the volatility in terms of the vapour pressure and the Henry constant.

There are no data provided regarding the hazard of Flazasulfuron to the ozone layer. The Ozone Depleting Potential (ODP) of Flazasulfuron has not been measured because this 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 half -life of was calculated as 0.636 hours, which corresponds to 0.053 days when a 12- hour day is considered and 0.027 days when a 24-hour day is considered. Any accumulation of Flazasulfuron in the troposphere is therefore unlikely to occur.

12.1.2 Comparison with the CLP 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 a danger to the structure and/or the functioning of the stratospheric ozone layer.

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).

Although no specific data have been provided for this hazard, considering the chemical structure and other available information on the physico-chemical properties, Flazasulfuron is not expected to be hazardous to stratospheric ozone.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified, data lacking.

13 ADDITIONAL LABELLING

No additional labelling is proposed.

14 REFERENCES

Full references given in the confidential CLH report.

Physico-chemical properties

Anonymous 47 (1996) SL-160 - MELTING POINT, BOILING POINT, RELATIVE DENSITY, PHYSICAL STATE, COLOR, ODOR, FLAMMABILITY, AUTOFLAMMABILITY Report No.: 4594-96-0188-AS-001, GLP, unpublished

Anonymous 48 (1993) FLAZASULFURON (SL-160) - COLOR, PHYSICAL STATE, ODOR, MELTING POINT, BULK DENSITY, OXIDATION- REDUCTION, IMPACT EXPLODABILITY Report No.: 4039-92-0496-AS-001, GLP, unpublished

Anonymous 49 (1993) FLAZASULFURON (SL-160) VAPOR PRESSURE Report No.: 4039-91-0399-AS-001, GLP, unpublished

Anonymous 50 (2014) SL-160: SURFACE TENSION Report No.: JSM0619 Huntingdon Life Sciences, Eye Research Centre, Eye, Suffolk, United Kingdom, GLP, unpublished

Anonymous 51 (1994) FLAZASULFURON (SL-160) SOLUBILITY Report No.: 4039-91-0400-AS-001, GLP, unpublished

Anonymous 52 (1996) DETERMINATION OF THE FLAMMABILITY OF SL-160 TECHNICAL Report No.: 623035, GLP, unpublished

Anonymous 53 (1998) EXPLOSIVITY OF SL-160 TECHNICAL Report No.: 715544, Not GLP, unpublished

Anonymous 54 (2014) STATEMENT RELATED TO THE OXIDISING PROPERTIES OF FLAZASULFURON Report No.: PP261-00015 Scientific Consulting Company, Bad Kreuznach, Germany, Not GLP, unpublished

Anonymous 55 (1992) FLAZASULFURON (SL-160) - DISSOCIATION CONSTANT Report No.: 4039-91-0404-AS-001, GLP, unpublished

Toxicology and metabolism

Toxicokinetics **(Absorption, Metabolism, Distribution and Elimination)**

Anonymous 1 (1994a) STUDY TO EVALUATE THE DISTRIBUTION AND EXCRETION OF ^{14}C SL-160(P) IN RATS (Doc. No: 5377-92-0329-AM-001-001). GLP, unpublished

Anonymous 2 (1995a) STUDY TO EVALUATE THE DISTRIBUTION AND EXCRETION OF ^{14}C SL-160(Pm) IN RATS (Doc. No: 5617-93-0034-AM-001). GLP, unpublished

Anonymous 3 (1995b) STUDY TO EVALUATE THE FARMACOKINETICS OF ^{14}C SL-160(P) IN THE BLOOD OF RATS (Doc. No: 5424-92-0330-AM-001) GLP, unpublished

Anonymous 4 (1995c) STUDY TO EVALUATE THE FARMACOKINETICS OF ^{14}C SL-160(Pm) IN THE BLOOD OF RATS (Doc. No: 5618-93-0035-AM-001). GLP, unpublished

Anonymous 5 (1995d) STUDY OF THE BILIARY EXCRETION OF RADIOLABEL FOLLOWING ORAL ADMINISTRATION OF ^{14}C SL-160(P) TO SPRAGUE-DAWLEY RATS (Doc. No: 5426-92-0332-AM-001). GLP, unpublished

Anonymous 6 (1995e) STUDY OF THE BILIARY EXCRETION OF RADIOLABEL FOLLOWING ORAL ADMINISTRATION OF ^{14}C SL-160(Pm) TO SPRAGUE-DAWLEY RATS (Doc. No: 5620-93-0037-AM-001). GLP, unpublished

Anonymous 7 (1996) PILOT STUDY TO EVALUTE THE EXCRETION OF RADIOLABEL FOLLOWING A SINGLE ORAL DOSE OF ^{14} C SL-160 TO RATS Rep. Doc. No: 5375-92-0271-AM-001. GLP, unpublished

Anonymous 8 (1995) STUDY TO IDENTIFY AND CHARACTERIZE THE METABOLITES OF (^{14}C)-SL-160 Doc. No 5427-92-0333-AM-001. GLP, unpublished

Anonymous 9 (1994b) STUDY TO EVALUATE THE DISTRIBUTION AND EXCRETION OF ^{14}C SL-160(P) FOLLOWING REPEATED ADMINISTRATION OF SL-160 TO RATS Report (Doc. No: 5425-92-0331-AM-001). GLP, unpublished

Anonymous 10 (1995f) STUDY TO EVALUATE THE DISTRIBUTION AND EXCRETION OF ^{14}C SL-160(Pm) FOLLOWING REPEATED ADMINISTRATION OF SL-160 TO RATS Report (Doc. No: 5619-93-0036-AM-001). GLP, unpublished

Anonymous 56 (2014) SL-160: RAT, DOG AND HUMAN LIVER MICROSOMAL METABOLISM Hls Study Number JSM0675. GLP, unpublished.

Acute toxicity

Anonymous 11 (1988a) TECHNICAL: ACUTE ORAL TOXICITY STUDY IN RATS Report No.: 87-0100 GLP, unpublished

Anonymous 12 (1988b) SL-160 TECHNICAL: ACUTE ORAL TOXICITY STUDY IN MICE Report No.: 87-0101

Anonymous 13 (1988) SL-160 TECHNICAL: ACUTE DERMAL TOXICITY STUDY IN RATS Report No.: 87-0102 GLP, unpublished

Anonymous 14 (1988) SL-160 TECHNICAL: ACUTE INHALATION TOXICITY STUDY IN RATS Report No.: 87-0106 GLP, unpublished

Anonymous 15 (1993a) PRIMARY DERMAL IRRITATION STUDY IN ALBINO RABBITS WITH SL-160 Report No.: 5490-92-0409-TX-1 GLP, unpublished

Anonymous 16 (1993b) PRIMARY EYE IRRITATION STUDY IN ALBINO RABBITS WITH SL-160 Report No.: 5489-92-0408-TX-1 GLP, unpublished

Anonymous 17 (1995) DERMAL SENSITIZATION STUDY (CLOSED PATCH REPEATED INSULT) IN GUINEA PIGS WITH SL-160 Report No.: 5488-92-0407-TX-1 GLP, unpublished

Anonymous 18 (1988) SL-160 TECHNICAL: DERMAL SENSITIZATION STUDY IN GUINEAN PIGS. MAXIMIZATION TEST Report No.: 96-0090 Not GLP, unpublished

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15 ANNEXES

Robust summaries of the studies are available in Volumes B.2, B.6, B.8 and B.9 of Renewal Assessment Report of the active substance Flazasulfuron on EFSA website (https://www.efsa.europa.eu/en/consultations/call/150929).