Committee for Risk Assessment

RAC

Annex 1

Background document

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

diflufenican (ISO); *N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide; 2′,4′-difluoro-2-(α,α,α-trifluoro-*m*-tolyloxy) nicotinanilide

EC Number: -
CAS Number: 83164-33-4

CLH-O-0000001412-86-285/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

13 June 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

diflufenican (ISO); *N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide; 2′,4′-difluoro-2-(α,α,α-trifluoro-*m*-tolyloxy)nicotinanilide

EC Number: **Not available**

CAS Number: **83164-33-4**

Index Number: **616-032-00-9**

Contact details for dossier submitter: **UK Competent Authority**
Chemicals Regulation Division
Health and Safety Executive
United Kingdom

Version number: 2 **Date: August 2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIFLUFENICAN (ISO); *N*-(2,4-DIFLUOROPHENYL)-2-[3-(TRIFLUOROMETHYL)PHENOXY]-3-PYRIDINECARBOXAMIDE; 2′,4′-DIFLUORO-2-(α,α,α -TRIFLUORO-*M*-TOLYLOXY) NICOTINANILIDE

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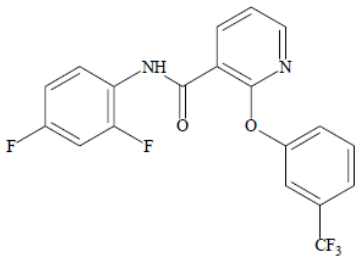
1 IDENTITY OF THE SUBSTANCE

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)	*N-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide*
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	*Diflufenican*
EC number (if available and appropriate)	*Not available (List number: 617-446-2)*
EC name (if available and appropriate)	*Not available*
CAS number (if available)	*83164-33-4*
Other identity code (if available)	*Not applicable.*
Molecular formula	$C_{19}H_{11}F_5N_2O_2$
Structural formula	
SMILES notation (if available)	*O=C(Nc1ccc(F)cc1F)c2cccnc2Oc3cccc(C(F)(F)F)c3*
Molecular weight or molecular weight range	*394.29 g/mol*
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*Not applicable.*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*Not applicable.*
Degree of purity (%) (if relevant for the entry in Annex VI)	*Min. 97.0 %*

1.2 Composition of the substance

Diflufenican does not contain any other constituents, isomers or additives.

There are a number of confidential impurities listed for diflufenican, none of which are relevant to the classification of the substance.

Self classification:

Aquatic Chronic 3; H412 - *applied by 161/189 notifiers at the time of submission of the CLH report. NB. 27/189 notifiers have also applied Aquatic Acute 1; H400 with an acute M-factor of 1000. 1/189 notifiers*

have notfied the following classification Skin Irrit. 2; H315, Eye Irrit. 2; H319 and STOT SE 3; H335 (Lungs)(Inhalation).

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIFLUFENICAN (ISO); *N*-(2,4-DIFLUOROPHENYL)-2-[3-(TRIFLUOROMETHYL)PHENOXY]-3-PYRIDINECARBOXAMIDE; 2′,4′-DIFLUORO-2-(α,α,α -TRIFLUORO-*M*-TOLYLOXY) NICOTINANILIDE

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	616-032-00-9	diflufenican (ISO); *N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide; 2′,4′-difluoro-2-(α,α,α-trifluoro-*m*-tolyloxy)nicotinanilide	617-446-2	83164-33-4	Aquatic Chronic 3	H412	No Pictogram or signal word.	H412	-	-	-
Dossier submitters proposal	616-032-00-9	diflufenican (ISO); *N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide; 2′,4′-difluoro-2-(α,α,α-trifluoro-*m*-tolyloxy)nicotinanilide	617-446-2	83164-33-4	**Modify:** Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	M = 1000 M = 100	-
Resulting Annex VI entry if agreed by RAC and COM	616-032-00-9	diflufenican (ISO); *N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide; 2′,4′-difluoro-2-(α,α,α-trifluoro-*m*-tolyloxy)nicotinanilide	617-446-2	83164-33-4	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	M = 1000 M = 100	-

Table 3: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is an existing entry in Annex VI of CLP (translated from Annex I of Dir 67/548/EEC) which includes classification with Aquatic Chronic 3; H412 only.

At the time of submission the substance is not registered under REACH.

RAC general comment
Diflufenican belongs to the class of anilide herbicides. It acts as a specific inhibitor of phytoene dehydrogenase, a key enzyme of carotenoid biosynthesis. It is used for the control of broadleaf weeds and a few annual grasses in winter cereals. The dossier submitter (DS) has reviewed the existing entry in Annex VI of CLP for diflufenican as a result of the renewal assessment under Regulation EC 1107/2009. Hence this proposal only addressed the reproductive toxicity and environmental hazards.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Diflufenican is an active substance in the meaning of Regulation EC 1107/2009 and therefore, according to Article 36(2) of the CLP further justification that action is required at a Community level is not required.

[A.] There is no requirement for justification that action is needed at Community level.

As a result of the renewal assessment under Regulation EC 1107/2009, it is appropriate to review the existing entry in Annex VI of CLP. This proposal is targeted to a consideration of the reproductive toxicity and environmental hazards. Studies investigating the repeated dose effects of diflufenican have been included as part of the assessment of reproductive toxicity and are provided as supporting information only.

5 IDENTIFIED USES

Diflufenican belongs to the class of anilide herbicides. It acts as a specific inhibitor of phytoene dehydrogenase, a key enzyme of carotenoid biosynthesis. Diflufenican is used for the control of broadleaf weeds and a few annual grasses in winter cereals.

6 DATA SOURCES

Conclusion regarding the peer review of the pesticide risk assessment of the active substance diflufenican (EFSA Scientific Report 2007)

Draft Renewal Assessment Report (dRAR) prepared by the UK Competent Authority (2018)

- Section B2 (Physical and chemical properties of the active substance)
- Section B6 (Toxicology and metabolism data)
- Section B8 (Environmental fate and behaviour)
- Section B9 (Ecotoxicology)

7 PHYSICOCHEMICAL PROPERTIES

Table 4: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	99.8 % Purity – white crystalline solid without odour 98.0 % Purity – beige powder with a phenol odour	Muehlberger (2002a)	OPPTS 830.6302, 830.6303, 830.6304
Melting/freezing point	159.5 °C (99.8 % Purity) 157.5 °C (98.0 % Purity)	Bascou (1998)	EEC A1 (DSC) GLP
Boiling point	No boiling point observed below 360°C	Bascou (1998)	EEC A2 (DSC) GLP Purity 99.5 %
Relative density	No data		
Vapour pressure	Vp (25°C) = 4.25 x 10-6 Pa Vp (35°C) = 8.19 × 10-6 Pa Vp (50°C) = 3.52 × 10-5 Pa	Cicotti (1992)	EEC A4 (gas saturation) GLP Purity 99.7 %)
Surface tension	71.46 mN/m	Bascou (1998)	EEC A5 (ring method) GLP Purity 98.1 %
Water solubility	Water pH 5.8 = 0.012 mg/L Buffer pH 4.5 = 0.005 mg/L Buffer pH 9.0 = 0.006 mg/L Water pH 6.89 <0.05 mg/L at 20°C	Bascou (1998)	EEC A6 (column elution method) GLP Purity 99.5 %
Partition coefficient n-octanol/water	log Pow = 4.2 at 20 °C	Muehlberger (2002b)	EEC A8 (HPLC method) OECD 117 GLP
Flash point	Not required as mp > 40°C		
Flammability	The substance melted on contact with the ignition source and no flame was observed. Experience in handling and use shows that the substance is not pyrophoric and does not emit flammable gases on contact with water.	François (1998)	EEC A10 GLP Purity 98.1 %
Explosive properties	No evidence of shock, friction or thermal sensibility.	François (1998)	EEC A14 GLP Purity 98.1 %
Self-ignition temperature	The substance melted when the over temperature	François (1998)	EEC A16 GLP

Property	Value	Reference	Comment (e.g. measured or estimated)
	reached 160°C. No evidence of self-ignition		Purity 98.1 %
Oxidising properties	Thet test substance/cellulose mixtures (10-90% test substance) ignited but failed to propogate combustion, or had slow buring rates (i.e., 100 mm in 6 mins). The buring rate of all test substance/cellulose mixtures was greater than the reference material.	François (1998) Bascou, J. P.; 1999	EEC A17 GLP Purity 98.1 %
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Due to the poor solubility of the molecule in water it was not possible to determine a dissociation constant. The structure of the molecule precludes any dissociation.	Muehlberger B., 2003c	OECD 112 GLP Purity 99.8 %
Viscosity	Not relevant.		

8 EVALUATION OF PHYSICAL HAZARDS

This endpoint has not been considered in this report.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

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

The pharmacokinetics of diflufenican has been extensively investigated in a number of studies using radiolabelled parent material where the label has been present at a number of sites on the multiple ring structure. Single dose studies have been carried out with 5 mg/kg ^{14}C-diflufenican labelled in the pyridine ring, difluorophenyl ring and the trifluoromethyl-phenyl ring and with 250 mg/kg ^{14}C-diflufenican labelled in the pyridine ring. Repeat dose studies have been carried out with 5 mg/kg ^{14}C-diflufenican labelled in the difluorophenyl ring.

Following oral administration, absorption from the gastro-intestinal tract is substantial and fairly rapid. On the basis of two single-dose studies in rats in which absorption over 48 hours was measured, an oral absorption value of 58% has been proposed. .

Radioactivity is distributed widely throughout the body and preferentially partitions into body tissues with a high adipose content. The highest levels of radioactivity were persistently detected in fat, skin and fur,

intestine and contents, ovaries, uterus and adrenals. Most tissue radioactivity levels decreased with time but there were indications that radioactivity accumulated in fat, gonads and uteri. Tissue accumulation of radioactivity was low with only a small proportion of the dose retained in the tissues at 168 hours (<0.67% of the administered dose). After single and repeated oral doses, accumulation of radioactivity also occurred in the whole blood and plasma of both sexes, with preferential accumulation in red blood cells.

The biotransformation of diflufenican is extensive with a high number of metabolic fractions in faeces, urine and bile. The principal metabolic reactions of diflufenican in the rat involve defluorination and subsequent hydroxylation, S-methylation and oxidation. In addition, glucuronide and possibly cysteine conjugation of the hydroxylated metabolites were observed in bile and faeces, respectively. Defluorinated metabolites represent <10% of the administered dose, which corrected for relative molecular weights equates to a systemically available fluoride dose of <1% w/w of the administered dose of diflufenican. Although the three-ring structure of diflufenican appears to remain largely intact during metabolism in the rat, the major metabolite AE B107137 was present in the urine of high and low dose animals which indicate cleavage of diflufenican at the amide bridge. *In vitro* comparative metabolism studies indicated that the general pattern of metabolism was qualitatively similar in the rat and humans, albeit with possible quantitative differences. No unique human metabolites > 1% were detected.

The predominant route of elimination of diflufenican and its metabolites is via the faeces, accounting for approximately 78-104% of an administered single low dose, of which biliary excretion accounted for approximately 30-48%. . After administration of a single low dose for 14 consecutive days, approximately 91% of the dose was eliminated in the faeces.

No significant differences in the *in vivo* pharmacokinetic or metabolic parameters were apparent between sexes.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

This endpoint has not been considered in this report.

10.2 Skin corrosion/irritation

This endpoint has not been considered in this report.

10.3 Serious eye damage/eye irritation

This endpoint has not been considered in this report.

10.4 Respiratory sensitisation

This endpoint has not been considered in this report.

10.5 Skin sensitisation

This endpoint has not been considered in this report.

10.6 Germ cell mutagenicity

This endpoint has not been considered in this report.

10.7 Carcinogenicity

This endpoint has not been considered in this report.

10.8 Reproductive toxicity

The reproductive toxicity of diflufenican has been investigated in a two-generation study in rats and several developmental toxicity studies in rat and rabbits. Additional information on possible effects of diflufenican on reproductive organs and tissues in repeated-dose toxicity studies is provided in section 10.10.

10.8.1 Adverse effects on sexual function and fertility

A two-generation study in rats by the oral route is available to investigate the effects of diflufenican on sexual function and fertility.

Table 2: Summary table of animal studies on adverse effects on sexual function and fertility

<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</th></tr>
<tr><td>Sprague Dawley rats
Dietary administration without dose adjustment
32 F0 / sex / group
28 F1 / sex / group
24 F2 / sex / group
OECD 415, 416 (1982)
GLP

Anon. 1985

&
Histopathology of the reproductive tract in F0 and F1A adult rats

Anon. 1987</td><td>0, 500, 2500, 12500 ppm
Diflufenican
Batch B20
Purity 98.1%
F0 animals (6 weeks of age) were treated for 10 weeks pre-mating until the end of lactation period for the first mating (F1A generation). For the second mating, animals were treated from 26 weeks of age throughout mating until</td><td>Mean achieved intakes of diflufenican for each generation:
<table><tr><th rowspan="3">Generation/Phase</th><th colspan="6">Mean diflufenican intake (mg/kg bw/day)</th></tr><tr><th colspan="3">Male</th><th colspan="3">Female</th></tr><tr><th>500 ppm</th><th>2500 ppm</th><th>12500 ppm</th><th>500 ppm</th><th>2500 ppm</th><th>12500 ppm</th></tr><tr><td>F0/pre-mating</td><td>35.5</td><td>175.6</td><td>888.0</td><td>41.9</td><td>206.1</td><td>1042.0</td></tr><tr><td>F1A/pre-mating</td><td>38.8</td><td>198.4</td><td>1035.0</td><td>47.3</td><td>223.7</td><td>1168.0</td></tr><tr><td>F2A/rearing</td><td>42.9</td><td>209.8</td><td>1044.0</td><td>50.8</td><td>244.2</td><td>1316.0</td></tr></table>
Parental toxicity
<u>F0 generation</u>
<u>Perinatal mortality</u>
500 ppm: 1 female on day 18
2500 ppm: 1 female at gestation day 22
12500 ppm: second mating; 2 females at post-partum day 8 (humane sacrifice) and gestation day 22
<u>Food consumption, bodyweight and bodyweight gain</u>
500 ppm: no effect observed.
2500 ppm (males & females): ↓ food consumption (≈ 10% reduction compared to controls) and body-weight gain (≈ 10% reduction compared to controls)
12500 ppm (males & females): ↓ food consumption (≈ 10-15% reduction compared to controls) and body-weight gain (≈ 15% reduction compared to controls)
<u>Organ findings</u>
Females only: ↓* relative thymus weight at all doses (dose-dependent reduction compared to controls ranging from 8% at 500 ppm down to 15% at 12500 ppm). No concurrent histopathology was conducted for F0 animals except for the reproductive tract.</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**
	the end of lactation period. F1A animals (4 weeks of age) were treated for 12 weeks during the pre-mating period until the end of lactation period for the first mating (F2A generation). For the second mating, animals were treated from 26 weeks of age from mating until the end of lactation period (F2B generation). F2A animals were treated from 4 weeks of age for 90 days before sacrifice. F2B animals were treated from 4 weeks of age for 14 days before	<u>Histopathology of the reproductive tract (at 0 and 12500 ppm only)</u> No adverse effects observed (males & females).
		<u>F1 generation</u> <u>Perinatal mortality</u> 500 ppm: 1 female 2500 ppm: none 12500 ppm: first mating: 2 females, at post-partum day 3 (humane sacrifice) and, post-partum day 8; second mating: 2 females at post-partum day 0 and post-partum day 0. <u>Food consumption, body-weight and body-weight gain</u> 500 ppm: no effect observed 2500 ppm (males & females): ↓ food consumption (≈ 10-20% reduction compared to controls) and body-weight gain (≈ 15% reduction compared to controls) 12500 ppm (males & females): ↓ food consumption (≈ 10-20% reduction compared to controls) and body-weight gain (≈ 10-15% reduction compared to controls) <u>Organ findings</u> 500 ppm: no adverse effect observed 2500 ppm: males ↓ relative (to brain) thymus weight (15.5% reduction compared to controls) 12500 ppm F1A males: ↑* relative (to bw) kidney weight (7% increase compared to controls), ↓ relative (to brain) thymus weight (25% reduction compared to controls); females: ↓ relative (to brain) thymus weight (11.5% reduction compared to controls) <u>Histopathology (lungs, thymus, liver, spleen, kidneys and reproductive tract)</u> 500 ppm: no treatment-related effects observed 2500 ppm: Dilated renal medullary collecting ducts (1/28 females); 12500 ppm: Dilated renal medullary collecting ducts (2/24 females); associated with mineral casts in one of the affected females. Minimal depletion of cortical tissue in thymus in 4/28 males and 6/24 females; associated with lower relative (to brain) thymic weights in both sexes. No adverse effects of treatment on the reproductive tract (males & females).
		Fertility
		<u>F0- & F1 generations</u> No treatment-related adverse effect observed.
		Offspring toxicity
		<u>F1 pups (excluding total litter loss)</u> <u>Litter findings</u> Dose-dependent decrease in mean pup weight observed in F1A & F1B from birth up to post-natal day 21 (weaning) 500 ppm: no adverse effects observed 2500 ppm: F1A ↓** & F1B ↓*** litter and mean pup weight Day 21 12500 ppm F1A ↓** & F1B ↓*** litter and mean pup weight Day 21; F1A ↑** cumulative pup loss/pup mortality (day 0-21)

<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</th></tr>
<tr><td></td><td>sacrifice.</td><td>
<table>
<tr><td></td><td colspan="4">F1 pups Litter data up to Day 21 –</td></tr>
<tr><td></td><td colspan="4">Dose (ppm)</td></tr>
<tr><td></td><td>Control</td><td>500</td><td>2500</td><td>12500</td></tr>
<tr><td>1st mating</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Litter size at birth</td><td>11.8</td><td>12.0</td><td>12.0</td><td>11.9</td></tr>
<tr><td>Mean pup weight at birth (g)</td><td>6.0</td><td>5.9</td><td>5.7*</td><td>5.2***</td></tr>
<tr><td>Cumulative pup loss (Day 0-21) %</td><td>3.4</td><td>5.6</td><td>6.4</td><td>11.2**</td></tr>
<tr><td>Litter wt. Day 21(g)</td><td>504.6</td><td>497.3</td><td>444.7***</td><td>408.0***</td></tr>
<tr><td>Mean pup wt. Day 21(g)</td><td>43.8</td><td>42.7</td><td>38.0***</td><td>34.8***</td></tr>
<tr><td>2nd mating</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Litter size at birth</td><td>10.7</td><td>11.8</td><td>10.2</td><td>10.3</td></tr>
<tr><td>Mean pup weight at birth (g)</td><td>6.1</td><td>5.9</td><td>5.8*</td><td>5.7**</td></tr>
<tr><td>Cumulative pup loss (Day 0-21) %</td><td>11.8</td><td>8.4</td><td>15.1</td><td>7.9</td></tr>
<tr><td>Litter wt. (g)</td><td>486.1</td><td>497.2</td><td>395.8**</td><td>377.3***</td></tr>
<tr><td>Mean pup wt. (g)</td><td>45.8</td><td>43.7</td><td>40.9**</td><td>38.4***</td></tr>
</table>
* p<0.05, ** p<0.01, *** p<0.001 (Kruskal-Wallis test intergroup comparison with the control)

<u>Organ findings – relative organ weight adjusted to bodyweight</u>

F1A young

500 ppm: females ↓* spleen (10% reduction compared to controls)
2500 ppm: spleen females ↓**(11% reduction compared to controls); males ↓* (10% reduction compared to controls)
12500 ppm: females ↓** spleen (14% reduction compared to controls) ↓* thymus (17% reduction compared to controls); males ↓* spleen (13% reduction compared to controls)
Note: Thymus weight change was dose-dependent in both sexes.

F1A adults

500 ppm: no effect observed
2500 ppm: no effect observed
12500 ppm: males ↑* kidney (7% increase compared to controls)

<u>F2 pups (excluding total litter loss)</u>

<u>Litter findings</u>
Dose-dependent decrease in mean pup weight observed at F2A & F2B from birth up to post-natal day 21 (weaning)
</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results

500 ppm: no adverse effects observed
2500 ppm: F2A & F2B ↓* mean pup weight Day 21
12500 ppm F2A ↓** litter weight ↓*** mean pup weight Day 21;
F2B ↓*** litter weight, ↓* mean pup weight Day 21, ↑** cumulative pup loss/pup mortality (day 0-21)

	F2 pups Litter data up to Day 21 –			
	Dose (ppm)			
	Control	**500**	**2500**	**12500**
1st mating				
Litter size at birth	11.0	11.7	12.0	10.5
Mean pup weight at birth (g)	5.8	5.8	5.6	5.4**
Cumulative pup loss (Day 0-21) %	7.3	5.7	4.9	13.0
Litter wt. (g)	459.7	482.1	455.5	369.8**
Mean pup wt. (g)	43.3	42.4	39.0*	36.0***
2nd mating				
Litter size at birth	12.4	12.3	12.4	9.5**
Mean pup weight at birth (g)	5.9	5.8	5.8	5.4**
Cumulative pup loss (Day 0-21) %	6.4	9.2	4.2	26.4**
Litter wt. (g) at Day 21	480.7	484.4	449.6	329.3***
Mean pup wt. (g) at Day 21	39.3	40.4	36.5*	35.3*

* p<0.05, ** p<0.01, *** p<0.001 (Kruskal-Wallis test intergroup comparison with the control)

Organ findings
F2A young
500 ppm: no adverse effect observed
2500 ppm: male: ↓** spleen (15% reduction compared to controls)
12500 ppm: male: ↑** liver (10% increase compared to controls) ↓** spleen (12% reduction compared to controls)

F2A adult
500 ppm: males ↑** kidney (9.3% increase compared to controls); females ↑** liver (12% increase compared to controls)
2500 ppm: males ↑* kidney (11% increase compared to controls); females ↑** liver (14% increase compared to controls)
12500 ppm: males ↑* kidney (8.4% increase compared to controls); females ↑** liver (20% increase compared to controls). Increase in liver was dose-dependent.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**
		F2B young 500 ppm: no adverse effect observed 2500 ppm: males ↑** liver (9% increase compared to controls) 12500 ppm: males ↑** liver (9% increase compared to controls) <u>Histopathology (lungs, thymus, liver, spleen, kidneys) F2A weanlings</u> 500 ppm: no treatment-related effects observed 2500 ppm: dilated renal medullary collecting ducts in 2/24 females associated with mineral casts in the dilated collecting ducts of one female. 12500 ppm: dilated renal medullary collecting ducts in 1/19 males and 2/17 females associated with mineral casts in all affected weanlings. Wedge-shaped areas of dilated cortical tubules were also observed in 2/19 male weanlings and 1/17 female weanlings.

Legend:
gd = gestation day; [c] ppd = postpartum day
Statistically significant at: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

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

A GLP and OECD guideline compliant oral two-generation study in the rat is available to inform on the toxicity of diflufenican to fertility and sexual function. Diflufenican was administered via the diet at dose levels that approached or exceeded the limit dose for this study protocol. No specific treatment-related clinical signs other than around parturition were seen in any animals in any generation. At 12500 ppm, there was an increase in deaths of females from the period just prior to parturition up to day 8 post-partum.

Parent findings

Bodyweight gain and food consumption

Both males and females demonstrated an overall reduction in food consumption at 2500 and 12500 ppm (≈ 10-15% reduction compared to controls). In the F0 adult animals, food consumption decreased during the pre-mating phase in both males and females from 2500 ppm. In the F1and F2 males a similar decrease in food consumption was seen in all treated groups compared to controls. From 2500 ppm for F1 and F2 females there was a decrease in food consumption to approximately 10-20% and 5-10% reduction compared to controls, respectively.
Body weight gain was also reduced in both males and females in all generations from 2500 ppm with a decrease ranging from 10-20% reduction compared to controls.

Mortalities

Nine females died or were killed prematurely during the study; information linked to each death is presented in the table below. Two of these were clearly incidental, whilst up to 6/7 female deaths were plausibly linked to dystocia (please refer to the paragraph ***Perinatal period findings*** for details).

Two males died during the study (a control male at week 30 in the F1A generation and one male at 500 ppm during week 29 of the F0 generation) but were not treatment related since there was no dose-response relationship.

Reproductive function

Mating performance and pregnancy rate were unaffected in any of the four pairings. The reproductive tracts of all females in the control and 12500 ppm groups were examined by standard histopathological techniques, and there was no indication of an overall effect on ovarian appearance or function. No treatment-related effects were observed in the males; over the two generations, the number of males which failed to induce pregnancy at either mating was 1, 2, 3 and 1 at 0, 500, 2500 and 12500 ppm respectively. At 2500 ppm (mid-dose level), two males demonstrated reduced spermatogenesis with reduced numbers of spermatozoa in the epididymides, but there was no such finding in the high-dose group. For all other animals failing to mate, histopathological examination of the reproductive tract showed them to be within normal limits.

Perinatal period findings

The duration of gestation was unaffected by administration of test material in any of the four pairings.

The majority of the dams found dead at or near parturition (5 out of 7 total) were found during delivery of the pups resulting from the second mating of the F0 or F1 adults, and 6 of the 7 animals were in the 12500 ppm dose group (equivalent to an intake of 1042 mg/kg bw/day in the F0 adults and 1168 mg/kg bw/day in the F1 adults). The study authors concluded that at 12500 ppm the relatively high number of deaths indicated a treatment-related effect associated with the added stress of parturition.

At the highest dose tested (12500 ppm), four out of the six dams that died had failed to deliver all their offspring; they were therefore clearly diagnosed with dystocia. The last two females may have also died as a consequence of dystocia. The first female was humanely sacrificed at PND 8 owing to poor condition linked to paralysis of the hind-limbs (female #235). This condition possibly resulted in a distended uterus which was observed at autopsy. Unfortunately no clear information is available to explain when the paralysis occurred, i.e., before, during or after parturition. On that basis the dossier submitter considers that dystocia cannot be ruled out because the paralysis could be linked to difficult parturition. The second female lacked evidence of difficult parturition (female #479). A single incidence of dystocia (incomplete parturition) occurred at the mid-dose level in the F0 generation but was not repeated in the F1 generation.

Macroscopic examinations of adult animals (F0, F1A and F2A) did not reveal any treatment-related findings.

The following table summarises the clinical findings of the dams that died or were prematurely sacrificed.

Table 6. Incidence of perinatal mortality and potential dystocia in F0 and F1 dams

Dose level / Dam ID	Time / cause of death / clinical signs	Dystocia diagnosis - parturition delayed or incomplete?
0ppm	-	0
500ppm		
F0 A		
172	Humanely sacrificed on first mating period day 18. Poor condition immediately prior to sacrifice with skeletal muscle, left dorsal abdominal cavity mass 12 x 12 x 7 mm, cut surface, pale, haemorrhagic tissue. Animal lethargic, weight loss 48 g over 2 days prior to sacrifice. Non-pregnant.	Death caused by poor clinical condition and dorsal injury → not dystocia
F1 A		
422	Right eye prominent and congested. Autopsy - right eye ruptured and haemorrhagic. Left lower molar, crown missing.	Death likely resulting from eye injury → not dystocia
	Dystocia total	0
2500ppm		
F0 B		
221	Found dead on day 22 of the second gestation. No clear cause of death. No delay in duration of gestation but parturition not completed as all 15 fetuses undelivered.	Parturition incomplete at death → **dystocia plausible**
	Dystocia total	1
12,500ppm		
F0 B		
235	Humanely sacrificed on PND 8. Poor condition linked to paralysis of the hind limbs. No delay in gestation, no undelivered fetuses. Litter all dead by PND 7, likely due to starvation.	Death likely resulting from hind limb paralysis → **equivocal link to dystocia but cannot be dismissed**
237	Found dead on day 22 of the second gestation. No clear cause of death. No delay in gestation but parturition not completed as all 14 fetuses undelivered.	No increase in duration of gestation; parturition incomplete at death → **dystocia plausible**
	Dystocia total	1
F1 A		
479	Found dead on PND 8 of the first litter. No adverse clinical signs prior to death. No undelivered fetuses, though only 2 born.	No increase in duration of gestation, parturition complete → not dystocia
459	Humanely sacrificed on PND 3. Duration of parturition extended; 6 fetuses delivered, 2 found undelivered at necropsy. Other findings included pallor, piloerection, and blood staining of the fur around the vaginal opening.	Parturition incomplete at death → **dystocia plausible**
	Dystocia total	1
F1 B		
474	Found dead PND 1 of the second mating. No adverse clinical signs prior to death. Parturition incomplete: 3 pups born, 12 undelivered	No increase in duration of gestation; parturition incomplete at death → **dystocia plausible**
478	Found dead PND 1 of the second mating. No adverse clinical signs prior to death. Parturition incomplete: 1 pup born, 13 undelivered	No increase in duration of gestation; parturition incomplete at death → **dystocia plausible**
	Dystocia total	2

PND = post-natal day

The body weight data shown in Table 7 and 8 for the F0 and F1 dams respectively clearly shows that body weight was notably decreased in a dose-dependent manner with a decrease of around 13-18% at 12500 ppm and approximately 6-14% at 2500 ppm during gestation and lactation and compared to controls. This is a treatment related effect which is maintained throughout gestation and lactation.

Table 7. Maternal body weight in F0 dams during gestation and lactation of the F1A and F1B litters, expressed as absolute values and as percentage of concurrent control group dams

		Diflufenican, dietary concentration in ppm							
		Gestation of F1A litter				**Gestation of F1B litter**			
Day		**0**	**500**	**2500**	**12500**	**0**	**500**	**2500**	**12500**
mg/kg bw/day		0.0	41.9	206.1	1042	--[a]	--	--	--
GD 0	g	284.0	277.8	260.3**	235.8**	327.1	305.5	295.2**	270.7**
	% control	--	*97.8*	*91.7*	*83.0*	--	*93.4*	*90.2*	*82.4*
GD 7	g	306.8	298.5	282.0**	260.3**	357.6	337.0	324.4**	296.2**
	% control	--	*97.3*	*91.9*	*84.8*	--	*94.2*	*90.7*	*82.5*
GD14	g	335.2	325.3	309.0**	287.3**	381.6	364.8	347.6**	319.2**
	% control	--	*97.0*	*92.2*	*85.7*	--	*95.6*	*91.1*	*83.4*
GD 17	g	361.5	354.6	335.2**	313.5**	407.5	390.5	374.6**	343.2**
	% control	--	*98.1*	*92.7*	*86.7*	--	*95.8*	*91.9*	*84.1*
GD 20	g	399.0	391.0	375.9*	353.7**	445.4	426.6	410.8*	376.8**
	% control	--	*98.0*	*94.2*	*88.6*	--	*95.8*	*92.2*	*84.5*
LD 0	g	319.7	309.2	297.5*	272.1**	361.1	345.5	329.3*	304.9**
	% control	--	96.7	*93.1*	*85.1*	--	*95.7*	*91.2*	*84.4*
LD 7	g	333.6	324.2	309.9**	2.93.7**	356.1	335.2	323.9**	304.7**
	% control	--	*97.2*	*92.9*	*88.1*	--	*94.1*	*91.0*	*85.6*
LD 14	g	332.3	319.4	305.9**	289.5**	358.9	339.3	330.9**	311.7**
	% control	--	*96.1*	*92.1*	*87.1*	--	*94.6*	*92.2*	*86.9*
LD 21	g	313.7	301.3	295.2*	280.5**	335.4	318.6	307.5**	294.7**
	% control	--	*96*	*94.1*	*89.4*	--	*95.0*	*91.7*	*87.9*

GD = gestation day; LD = lactation day
Statistically significant at: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 8. Maternal body weight in F1 dams during gestation and lactation of the F2A and F2B litters, expressed as absolute values and as percentage of concurrent control group dams

		Diflufenican, dietary concentration in ppm							
		Gestation of F2A litters				**Gestation of F2B litters**			
Day		**0**	**500**	**2500**	**12500**	**0**	**500**	**2500**	**12500**
mg/kg bw/day		0.0	47.3	223.7	1168	--[a]	--	--	--
0	g	281.0	272.5	242.3**	230.9	314.4	312.2	273.5**	263.1**
	% control	--	*97.0*	*86.2*	*82.0*	--	*99.3*	*87.0*	*83.7*
7	g	301.1	293.8	263.5**	252.6**	338.1	332.9	294.9**	283.9**
	% control	--	*97.6*	*87.5*	*83.7*	--	*98.5*	*87.2*	*84.0*
14	g	328.4	323.1	291.6**	280.2**	366.8	363.3	324.1**	311.0**
	% control	--	*98.4*	*88.8*	*85.0*	--	*99.0*	*88.3*	*84.8*
17	g	353.2	348.2	318.5**	303.3**	393.6	389.9	349.8**	339.2**
	% control	--	*98.6*	*90.2*	*85.4*	--	*99.1*	*88.9*	*86.2*
20	g	389.2	384.2	357.7**	342.2**	434.2	429.9	390.2**	378.4**
	% control	--	*98.7*	*91.9*	*87.4*	--	*99.0*	*89.9*	*87.2*
LD 0	g	312.2	297.6	275.3**	265.3**	348.9	343.3	310.3**	297.8**
	% control	--	95.3	*88.2*	*85.0*		*98.4*	*88.9*	*85.4*
LD 7	g	322.3	313.4	287.9**	281.5**	355.1	348.9	319.2**	313.8
	% control	--	*97.2*	*89.3*	*87.3*		*98.2*	*89.9*	*88.4*
LD 14	g	326.9	319.2	299.5**	286.8**	360.3	346.2	322.0**	317.5**
	% control	--	*97.7*	*91.6*	*87.7*		*96.1*	*89.4*	*88.1*
LD 21	g	312.5	302.0	285.4**	283.7**	340.0	331.3	305.5**	306.4**
	% control	--	*96.6*	*91.3*	*90.8*		*97.4*	*89.8*	*90.1*

GD = gestation day; LD = lactation day
Statistically significant at: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Historical control data for dystocia in the rat

The study sponsor compiled in-house data collected from 3 preliminary one-generation studies and 7 definitive two-generation studies performed on their behalf in the same laboratory using the same strain of animals between 1981 and 1988 (the timeframe is relevant as the study was conducted in 1985). Dystocia was identified in control animals in 2/10 studies (a single case in a 1981 study and two cases in a 1983 study). The animal supplier indicated an incidence of perinatal maternal mortality in their breeding colonies of approximately 0.2-0.5%.

The table below presents the incidence of dystocia identified in the present study for comparison.

Table 9: Incidence of diagnosed dystocia in F0 and F1 dams in the study

Dystocia / litters / mated female rats	**Diflufenican, dietary concentration in ppm**			
	0	**500**	**2500**	**12500**
F0 first mating **% incidence of dystocia**	0/32/32 0	0/30/32 0	0/31/32 0	0/29/32 0
F0 second mating **% incidence of dystocia**	0/25/32 0	0/21/31 0	1/23/32 4.3	2/23/32 8.7
F1 first mating **% incidence of dystocia**	0/26/28 0	0/25/28 0	0/24/28 0	1/24/28 4.2
F1 second mating **% incidence of dystocia**	0/26/28 0	0/26/28 0	0/26/28 0	2/22/26 9.1
Total number **(%) incidence of dystocia in pregnant rats across/ group**	0/109/120 (0)	0/102/120 (0)	1/104/120 (1)	5/98/120 (5.1)

% incidence of assumed dystocia in litters (pregnant rats)

Organ weight findings and histopathology

Changes in relative organ weights included a general decrease in thymus weight in females in the F0 generation at all dose levels. In the F1 generation minimal depletion of cortical tissue in thymus was reported in 4/28 males and in 6/24 females at 12500 ppm. This change was associated with lower relative (to brain) thymus weights in both sexes [25 and 12% reduction for males and females respectively (compared to controls)] of this dose group.

A number of treatment-related kidney changes were apparent in the parent generations. Statistically significant increases in relative kidney weights were seen in adult males in the F1A generation at 12500 ppm only. Following histopathological examination of the kidney, dilated medullary collecting ducts was reported in 2/24 females at 12500 ppm, which was associated with mineral casts in one of the affected females, and in 1/28 F1A females at 2500 ppm..

Most of the F0 and F1 adult animals treated with diflufenican at 12500 ppm did not have any microscopic findings associated with the reproductive tract (Offer, J. M.; 1987). In males 2/ 32 in each group of the F0 generation were found to have minimal (control) or minimal to moderate (12500 ppm) atrophy of seminiferous tubules. One animal in the 12500 ppm group displayed reduced spermatozoa in the epididymides, and one was found to have an epididymal spermatocele granuloma. However, no fertility parameters were affected in this study.

Litter findings

The incidence of total litter loss was generally low: the overall incidence from the four matings was 0, 0, 0, 0 and 1, 0, 0, 1 at 0, 500, 2500 and 12500 ppm in the 1st and 2nd matings of the F0 generation and 1, 4, 0, 3

and 0, 0, 1, 1 at 0, 500, 2500 and 12500 ppm in the 1st and 2nd matings of the F1A generation. The highest single incidence occurred at 500 ppm at the first mating of the F1A generation (4 losses).

At birth a dose-dependent decrease in mean pup weight was observed and the differences attained statistical difference for all matings at 12500 ppm and generally but to a lesser extent at 2500 ppm compared to control groups. This finding was associated with a statistically significant decrease in mean litter weight in 2/4 matings. Litter size at birth was unaffected.

Pup weight gain from birth up to day 21 was statistically significantly lower at 12500 ppm, and to a lesser extent at 2500 ppm, in both generations. Less consistent litter finding noted at 12500 ppm was a statistically significant increase in pup mortality (expressed as cumulative pup loss between day 0-21) at the first mating of the F0 generation and second mating of the F1 generation. However, cumulative pup loss at day 21 was generally not dose-dependent across the 4 matings and inconsistently reached statistical significance at 12500 ppm in 2/4 matings compared to controls.

Overall the litter effects observed at 12500 ppm are likely to be the consequence of the markedly lower pup weight observed at birth compared to controls, resulting in a marked reduction in the ability of litters to thrive up to weaning (day 21). It is to be noted that maternal weight was affected in a dose-dependent manner during gestation. Despite their initial reduced bodyweight at birth, the surviving pups at 12500 ppm gained weight in a similar pattern to the other dose groups up to weaning (Table 10).

Table 10: Pup bodyweight development up to weaning (day 21)

Generation	Day	Bodyweight						
		0ppm	500ppm		2500ppm		12500ppm	
		(g)	(g)	% control	(g)	% control	(g)	% control
F1A	0	6.0	5.9	98.3	5.7*	95.0	5.2***	86.7
	4	9.5	9.3	97.8	8.7*	91.6	8.2***	86.3
	8	15.3	15.4	100.7	14.2*	92.8	13.5***	88.2
	12	22.8	22.9	100.4	21.2**	93.0	20.1***	88.2
	21	43.8	42.7	97.5	38.0***	86.8	34.8***	79.5
F1B	0	6.1	5.9	96.7	5.8*	95.1	5.7**	93.4
	4	9.7	9.3	95.9	9.2	94.8	9.2	94.8
	8	15.8	15.4	97.5	15.3	96.8	14.9	94.3
	12	23.2	22.7	97.8	22.2	95.7	21.8	94.0
	21	45.8	43.7	95.4	40.9**	89.3	38.4***	83.8
F2A	0	5.8	5.8	100.0	5.6	96.6	5.4**	93.1
	4	8.7	9.0	103.4	8.3	95.4	7.7**	88.5
	8	14.9	15.1	101.3	13.9	93.3	12.9*	86.6
	12	22.6	22.6	100.0	21.4	94.7	20.3*	89.8
	21	43.3	42.4	97.9	39.0*	90.1	36.0***	83.1
F2B	0	5.9	5.8	98.3	5.8	98.3	5.4**	91.5
	4	8.4	8.4	100.0	8.5	101.0	7.2**	85.7
	8	13.6	14.0	102.9	13.4	98.5	11.4**	83.8
	12	20.6	21.3	103.4	20.3	98.5	18.3*	88.8
	21	39.3	40.4	102.8	36.5*	92.9	35.3*	89.8

* significant at $p<0.05$
** significant at $p<0.01$
*** significant at $p<0.001$

There were no treatment-related effects on sex ratio or pre-weaning development. The incidence of structural anomalies recorded at autopsy of excess F1 and F2 offspring did not indicate any adverse relationship to dietary concentration of diflufenican.

Offspring findings

Organ weight changes and histopathology

Changes in relative organ weights included a decrease in thymus weight in F1A weanlings at 12500 ppm. Spleen weights were reduced in male F1A and F2A weanlings at 2500 - 12500 ppm, and in female F1A weanlings at all concentrations of test material. However, no histopathology findings were associated with these organ weight changes.

Similarly, young males in the F2A and F2B generations demonstrated increased liver weights at the higher doses (increases less than 10% over controls). Adult F2A female animals showed significant liver weight increases in all treatment groups, but the control value appears to be low in this group (10-20% increase compared to controls). These findings were not accompanied by microscopic changes in the liver.

A number of treatment-related kidney changes were apparent in the F2A weanlings. These included dilated collecting ducts in 2/17 females and 1/19 males at 12500 ppm; this was associated with mineral casts in all weanlings. Wedge-shaped areas of dilated cortical tubules were also observed in 2/19 male weanlings and 1/17 female weanlings. At 2500 ppm dilated medullary collecting ducts were observed in 2/24 weanling females.

No relevant organ weight or microscopic changes were observed at 500 ppm.

Overall conclusion

Diflufenican was administered in the diet at fixed concentrations of 0, 500, 2500 and 12500 ppm throughout. Among male and female adults an overall reduction in food consumption and bodyweight gain at 2500 and 12500 ppm was demonstrated. At 12500 ppm an increased incidence of mortality among females in the period just prior to parturition up to post-partum day 8 was observed. This dystocia effect is considered to be treatment-related. All the other fertility parameters investigated were unaffected by treatment at all doses. Other findings of note were a decrease in thymus weight associated at top dose with depletion of thymic cortical tissue in occasional animals, an increase in liver weight adjusted for bodyweight and occasional renal histopathology changes were seen in adults at 12500 ppm. However, no relevant microscopic findings were found in the reproductive tract and no notable effects were associated with the endocrine system. Diflufenican did not affect the reproductive tract or the endocrine system in the short-term and long-term studies available for diflufenican (see Section 10.10 for details).

Dystocia is a disruption in the normal progression of labour; it is a heterogeneous condition with multiple potential causes including maternal toxicity, hormone disruption, abnormalities in the maternal reproductive tract, malformed/large fetus or uterine inertia.

Some of the main observations that can lead to a diagnosis of dystocia are delayed or incomplete parturition or prolonged, difficult birth. In this present case, there was no delay in the onset of parturition as the duration of gestation was not affected, and the delivered pups weighed less than those in the control groups. The available data does not allow a conclusion to be made on a possible prolonged, difficult birth (since rats often deliver during the night, it can be difficult to detect such an effect). However, incomplete parturition (undelivered pups at necropsy) was reported for six cases of assumed dystocia: a single occurrence at 2500 ppm and five occurrences at 12500 ppm (Table 10.10.2.1).

In this study, dystocia manifested as perinatal death of several dams with no or incomplete delivery of their litters. The cases reported for the highest dose tested were spread across 2 generations and 3 matings ($F0_B$, $F1_A$ and $F1_B$); i.e., 5/98 pregnant animals (5.1%), with a maximum incidence of 2/22 pregnant animals ($F1_B$). A single incidence of dystocia occurred at 2500 ppm (equivalent to ~ 200 mg/kg bw/day) in the F0 generation but was not repeated at F1 and was most likely a spontaneous occurrence; the available historical control data confirm that dystocia can occur spontaneously (at up to 0.5%). In general, however, dystocia is generally seen as a rare event in the rat.

During gestation the mean bodyweight of dams at F0 was reduced by 11-17% while for F1 dams it was reduced by 10-18% at 12500 ppm compared to control groups (Table 10.10.2.2. and 3). This is an indication of maternal toxicity, however no concomitant common clinical signs were observed for the females affected by dystocia or in all female groups in general. Unfortunately, it is not possible to assess the maternal toxicity of the affected dams on an individual basis, because the individual bodyweight data during gestation for dams which died were not reported. The short- and long-term toxicity studies available for diflufenican in the rat (13-weeks, 104-week studies), for which female systemic toxicity findings are relevant to the findings seen in the 2-generation study, provide some additional information; in general in these studies, females that showed similar bodyweight reduction compared with controls did not show any concomitant severe clinical / systemic toxicity.

Among the litter findings, a slight, dose-dependent decrease in mean pup weight was observed at birth and during pup rearing. The differences attained statistical difference for all matings at 12500 ppm and generally, but to a lesser extent, at 2500 ppm compared to control groups. The treatment at 12500 ppm was associated with a marked reduction in ability of litters to thrive up to weaning stage: the most consistent effects were observed on litter and mean pup weight accompanied on some occasions with increased pup deaths. Since the decreased birth-weight of pups occurred only in conjunction with decreased maternal body-weights, the dossier submitter concludes that this finding was secondary to maternal toxicity, not a specific effect on development. No adverse effects were observed on sex ratios, the stages of pre-weaning development or the incidences of anomalous pups. Some reduction in mean thymus or spleen weight and increase in liver weight were noted at 12500 and 2500 ppm in some batches of weanlings (consistent with findings of systemic toxicity in adults in this and the repeated-dose toxicity studies). Treatment-related renal effects were observed in occasional weanlings at 12500 ppm only, which was also consistent with parental effects. No adverse effects were noted on the reproductive tract.

10.8.3 Comparison with the CLP criteria

In a GLP and OECD-guideline-compliant oral two-generation study in the rat, diflufenican was administered via the diet up to dose levels approaching or exceeding the limit dose for this study protocol. The only observed effect that was potentially relevant to classification for adverse effects on sexual function and fertility was dystocia in a small number of animals in the high-dose group. Dystocia is listed in the CLP guidance as an effect that might lead to a classification for sexual function and fertility, since it is an adverse effect on pregnancy outcomes.

Diflufenican is not a known or presumed human reproductive toxicant; therefore, classification in category 1A for reproductive toxicity is not appropriate.

For classification in category 1B, animal data shall provide clear evidence of an adverse effect on sexual function or fertility in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on fertility is considered not to be a secondary non-specific consequence of other toxic effects. In the case of diflufenican, a low incidence of dystocia at very high doses (above the limit dose) was observed together with maternal toxicity. The dossier submitter considers that this does not provide clear evidence of an adverse effect, and therefore proposes that classification in category 1B is not appropriate.

Substances are classified in category 2 when there is some evidence from humans or experimental animals of an adverse effect on sexual function or fertility, and where the evidence is not sufficiently convincing to place the substance in category 1. 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.

In support of classification in category 2, the cases of dystocia appeared to be related to exposure to diflufenican: a small number of animals in the high-dose group were affected across generations and matings. A single occurrence in the mid-dose group, in the F0 generation, could not be definitely attributed to treatment; since this event also occurs in untreated rats, as shown by the historical control data, the dossier submitter concludes that this case was possibly incidental.

In support of no classification, the incidence of treatment-related dystocia was low and only occurred at a dose that exceeded the limit dose. Maternal toxicity was evident at this dose: body-weights of the dams were up to 17% lower than the control animals throughout the study. Furthermore, there was no evidence from the reproductive toxicity or repeated-dose toxicity studies that diflufenican acted through a specific mode-of-action that might result in dystocia; for example, it showed no endocrine disruption potential, no abnormalities were detected in the female reproductive tract, and the foetuses were not large or malformed. It seems most likely, therefore, that dystocia resulted from non-specific toxicity of the dams, not from a specific effect on reproduction. There were no other effects on reproduction or fertility in this study.

Overall, the dossier submitter considers that the adverse effect on pregnancy outcomes (dystocia) was most likely to be a secondary, non-specific consequence of other toxic effects. In particular, it is noted that this effect occurred only in dams that received a dose of diflufenican exceeding the limit dose of 1000 mg/kg bw/day recommended in the relevant OECD test guideline, which resulted in maternal toxicity. Moreover, there is no substantial evidence to show that diflufenican displays any specific effects on reproduction in the absence of maternal toxicity. Lastly, there is no evidence from the investigations into reproductive toxicity nor the repeated-dose toxicity studies that treatment with diflufenican affects the reproductive tract, the endocrine system or other systems that might be involved in parturition in rats..

In conclusion, the findings described in this study do not provide clear evidence of an effect on sexual function or fertility in the absence of other toxic effects to merit classification. Therefore, the dossier submitter proposes not to classify diflufenican for adverse effects on sexual function and fertility.

Conclusions on classification and labelling:

Not classified – Conclusive but not sufficient for classification

10.8.4 Adverse effects on development

With regards to the assessment of developmental toxicity of diflufenican, three developmental toxicity studies (oral route) are available, two conducted in the rat and one in the rabbit.

Table 11: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**
Oral gavage OECD 414 (1981) Sprague Dawley rats Pregnant females	Diflufenican Batch No. B20 Purity 98.1% 0, 50, 500 and 5000 mg/kg bw/day Administered	**Maternal toxicity** <u>Clinical signs</u> Dose-related ↑salivation (post-dosing); not seen beyond the last dosing day Pale faeces in high dose animals over days 7 to 16. No treatment-related deaths. <u>Food consumption / bodyweight gain</u> ≥ 500 mg/kg bw/day: dose-related ↓bodyweight gain throughout treatment.

<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</th></tr>
<tr><td>25 / group
Anon. 1984a</td><td>by gavage at dose volume of 2 ml/100g bw
Treated between days 6 and 15 of pregnancy</td><td>Food consumption at 5000 mg/kg bw/day slightly lower than controls (≈10% reduction compared to controls) (days 6 - 10).

<u>Gross necropsy findings</u>
None.

Developmental toxicity
<u>Litter findings</u>
No statistically significant differences in litter size, litter weight or pre- or post-implantation losses between the treated and the control groups.
Sex ratio unaffected by treatment.
<u>Malformation incidence</u>
No dose-related increase and no pattern to the malformations found
<u>Anomalies incidence</u>
<u>Visceral anomalies</u>
Dose-dependent mean increase (%)observed although no obvious pattern or relationship between the anomalies seen. No historical control data available.
<u>Skeletal anomalies</u>
No treatment-related increase observed.
<u>Sternebral and additional ribs</u>
Not affected by treatment.

<u>Group mean incidence of malformations and anomalies</u>
<table>
<tr><th colspan="4">Group (mg/kg bw/d)</th><th>Control</th><th>50</th><th>500</th><th>5000</th></tr>
<tr><td colspan="4">N° of litters</td><td>19</td><td>21</td><td>19</td><td>21</td></tr>
<tr><td rowspan="9">N° of pups with</td><td rowspan="3">Malformations</td><td colspan="2">Examined</td><td>199</td><td>205</td><td>195</td><td>207</td></tr>
<tr><td colspan="2">Total (N)</td><td>3</td><td>5</td><td>1</td><td>3</td></tr>
<tr><td colspan="2">Mean (%)</td><td>1.5</td><td>2.6</td><td>0.7</td><td>1.4</td></tr>
<tr><td rowspan="6">Anomalies</td><td rowspan="3">Skeletal</td><td>Examined</td><td>98</td><td>101</td><td>97</td><td>102</td></tr>
<tr><td>Total (N)</td><td>13</td><td>16</td><td>12</td><td>14</td></tr>
<tr><td>Mean (%)</td><td>14.3</td><td>16.1</td><td>13.9</td><td>13.3</td></tr>
<tr><td rowspan="4">Visceral (Wilson technique)</td><td>Examined</td><td>98</td><td>99</td><td>97</td><td>102</td></tr>
<tr><td>Total (N)</td><td>3</td><td>5</td><td>5</td><td>10</td></tr>
<tr><td>Mean (%)</td><td>3.2</td><td>4.7</td><td>5.8</td><td>9.6</td></tr>
<tr><td>N° litter affected</td><td>2</td><td>5</td><td>5</td><td>7</td></tr>
</table>
</td></tr>
<tr><td>Oral gavage
OECD 414 (1981)
Wistar rats</td><td>Diflufenican
Batch No.7
Purity 98.8%
0, 250, 500 and</td><td>Maternal toxicity
<u>Clinical signs</u>
None observed.
No treatment-related deaths reported.</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**
Pregnant females 28/group Anon. 2002	1000 mg/kg bw/day Administered by gavage at dose volume of 5 ml/kg bw Treated between days 6 and 15 of pregnancy	Food consumption / bodyweight gain Comparable to the vehicle control group values at all doses. Maternal parameters Comparable mean number of corpora lutea, implantations, early and late resorptions, pre- and post-implantation loss and dams with any/all resorptions to the respective vehicle control values in all treatment groups. Gross necropsy findings No treatment-related findings observed. **Developmental toxicity** Litter findings No statistically significant differences in litter size, litter weight between the treated and the control groups. Sex ratio unaffected by treatment. External observations No treatment-related observations found. Visceral observations No visceral anomalies observed. Skeletal observations No major malformations observed. No treatment-related effect on the normal variant parameters reported. No increase in the incidence of minor anomalies observed compared to control.
Oral gavage OECD 414 (1981) New Zealand White rabbits Pregnant females Anon. 1984b	Diflufenican Batch No. B20 Purity 98.1% 0, 50, 350 and 2500 mg/kg bw/day The top dose greatly exceeds the limit dose of 1000 mg/kg bw/day recommended in the current OECD guideline. Administered at dose volume of 10 ml/kg bw Treated	**Maternal toxicity** Clinical signs No treatment-related deaths. 2500 mg/kg bw/day: pale faeces (up to day 19), red discoloured urine (11 / 16 animals towards end of treatment, persisting few days post dosing period), reduced faecal output associated with reduced food consumption. 350 mg/kg bw/day: red discoloured urine (3/13 animals) at immediate post-dosing period. Food consumption / bodyweight gain 2500 mg/kg bw/day: both parameters clearly reduced throughout the treatment period but recovered once treatment ceased. 50-350 mg/kg bw day: food consumption lower than controls however not considered treatment related because the reduction was observed during the pre-treatment period and remained as such throughout treatment. Maternal parameters One dam at 350 mg/kg bw/day aborted its litter on day 24 (10 abortion sites in the uterus). No treatment-related increase in post-implantation losses and litter size.

<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</th></tr>
<tr><td></td><td>between days 6 and 18 of pregnancy</td><td>
<u>Gross necropsy findings</u>
No treatment-related findings.
Offspring toxicity
<u>Litter findings</u>
No treatment-related effect on litter weight, mean foetal weight or sex ratio.
<u>Skeletal observations</u>
No treatment-related findings
<u>Sternebral and additional ribs</u>
Variant sternebrae incidence not increased by treatment.
↑ Extra ribs incidence in all treated groups in a dose-related pattern (statistically significant at 2500 mg/kg bw/day). However, values were within historical control data range provided. Incidence in control animals relatively low. Findings associated with clear maternal toxicity.
<u>Incidence of extra ribs and sternebra variants</u>
<table>
<tr><td>Dose mg/kg bw/</td><td></td><td rowspan="2">Fetuses examined</td><td colspan="2">12 ribs</td><td colspan="2">13 ribs</td><td colspan="2">Normal sternebrae</td><td colspan="2">Variant sternebrae</td></tr>
<tr><td>day</td><td>N</td><td>Total</td><td>Mean %</td><td>Total</td><td>Mean %</td><td>Total</td><td>Mean %</td><td>Total</td><td>Mean %</td></tr>
<tr><td>0</td><td>12</td><td>96</td><td>80</td><td>81.0</td><td>16</td><td>19.0</td><td>66</td><td>71.7</td><td>30</td><td>28.3</td></tr>
<tr><td>50</td><td>16</td><td>111</td><td>74</td><td>63.6</td><td>37</td><td>36.4</td><td>89</td><td>85.6</td><td>22</td><td>14.4</td></tr>
<tr><td>350</td><td>13</td><td>97</td><td>65</td><td>60.9</td><td>32</td><td>39.1</td><td>73</td><td>78.0</td><td>24</td><td>22.0</td></tr>
<tr><td>2500</td><td>16</td><td>124</td><td>73</td><td>57.8</td><td>51</td><td>42.2*</td><td>114</td><td>92.5</td><td>10</td><td>7.5</td></tr>
</table>
* p<0.05
Historical control data for extra ribs:
N = 21 rabbit teratology studies (Jan 1983 – Jan 1984 from same laboratory)
Mean incidence = 34.3%; range 13.8 – 50%
</td></tr>
</table>

Legend
bw = bodyweight
Statistically significant at: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

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

<u>In the rat</u>

In a 1984 GLP study in the rat, the only clinical sign observed was a dose-related incidence of increased salivation in the post-dosing period. This sign was not seen beyond the last day of dosing, but was accompanied by brown facial staining on many occasions. These signs are considered to be related to gavage dosing with the test material suspensions. Pale faeces were also recorded in high dose animals over days 7 to 16.

Food consumption was slightly lower than controls (≈10% reduction compared to controls) in high dose animals over days 6 to 10. Bodyweight gains were lower than controls in a dose-related pattern at 500 and 5000 mg/kg bw/day, starting from the commencement of treatment on day 6 and persisting through the treatment period (gestation day 15). These effects on bodyweight gains showed at least partial recovery after the cessation of treatment.

There were no statistically significant differences in litter size, litter weight or pre- or post-implantation losses between the treated and the control groups. Sex ratio was not affected by treatment, and there were no gross necropsy findings in dams. The number of young with malformations (between 1 and 5 per group) did not indicate any teratogenic effect (there was no dose-related increase and no pattern to the malformations found). There was no increase in skeletal anomalies in treated animals of any group. The number of young with visceral anomalies was higher than controls in all treated groups in a dose-related pattern (3.2% in controls rising to 9.6% at the high dose level), but there was no obvious pattern or relationship between the anomalies seen. At 50 and 500 mg/kg bw/day the incidence of visceral findings is considered comparable to controls with only 2 additional foetuses affected in each dose group (n = 5/group) compared with controls (n = 3). The number of affected litters for visceral anomalies was 2, 5, 5 and 7 litters at 0, 50, 500 and 5000 mg/kg bw/day respectively. The incidence of foetuses with additional ribs or sternebral abnormalities was not affected by treatment.

In the second GLP- and OECD-compliant study conducted in the rat (Anon. 2002), no parental deaths or clinical signs were observed in any of the treatment groups. The body weight and body weight gain, as well as the food intake during the different phases of gestation in all treatment groups, were comparable to the vehicle control group values. The maternal parameters such as the mean number of corpora lutea, implantations, early and late resorptions, pre- and post-implantation loss and dams with any/all resorptions were comparable to the respective vehicle control values in all the treatment groups. There were no treatment-related gross visceral lesions in the rats sacrificed at term.

The litter data parameters such as mean litter size, number and weight of the fetuses and sex ratio of the treatment groups were statistically comparable to the respective vehicle control values. There were no treatment-related external observations (including major malformations) in any diflufenican-exposed group. The normal skeletal variant parameter changes observed, such as for delayed skeletal ossification and incomplete/poor ossification did not reveal any clear biological significance or treatment-related pattern. The incidences of minor skeletal anomalies observed in one or more of the treatment groups were comparable to the concurrent vehicle control values or comparable to the historical control data. Overall, no concern has been raised from the skeletal findings reported in this study. There were no statistically significant or dose-related increases in visceral malformations or minor anomalies compared to the vehicle control values. The normal variant visceral parameters considered in the study were also comparable to the vehicle control values. In conclusion, this study provided no evidence of developmental toxicity in rats when diflufenican was administered orally up to the limit dose.

In the rabbit

In a 1984 GLP study conducted in the rabbit, in which diflufenican was administered orally (by gavage) at doses of 0, 50, 350 and 2500 mg/kg bw/d, there were no treatment-related deaths. Clinical signs in the high dose group included pale faeces (for most animals during most of the treatment period, persisting as far as day 19) and red discoloured urine (in 11 out of 16 animals towards the end of the treatment period, persisting a few days into the post-dosing period). Reduced faecal output was also frequently recorded (associated with reduced food consumption). Food consumption was clearly reduced at 2500 mg/kg bw/day throughout the treatment period but recovered once treatment ceased. Bodyweight gain was markedly lower than controls during the treatment period at 2500 mg/kg bw/day, especially early in the treatment period, but recovered once treatment ceased. A single animal aborted on day 24, at 350 mg/kg bw/day. There were no treatment-related gross necropsy findings in dams.

There was no treatment-related effect on post-implantation losses, litter size, litter weight, mean foetal weight or sex ratio. The incidence of malformations (1 or 2 in each group) did not indicate a teratogenic effect, and the pattern of anomalies identified by gross dissection or skeletal examination did not indicate any effect of treatment. The incidence of variant sternebrae was not increased by treatment. The incidence of the common variation rudimentary extra ribs was higher than controls in all treated groups, in a dosage-related pattern. The difference from controls was statistically significant at the high-dose level. However, the values in all treatment groups were well within the historical control range, whereas the incidence in control animals was relatively low. Therefore, this finding does not present clear evidence of a treatment-related effect.

Overall conclusion

In the two rat developmental toxicity studies, diflufenican was administered orally at doses that equalled or exceeded the limit dose for the test guideline. In the first study there were clear signs of maternal toxicity at 5000 mg/kg bw/day; litter size and litter weight were also slightly lower at the high dose level, presumably as a consequence of the maternal toxicity. Despite this, no clear evidence of developmental toxicity was identified even at the excessively high dose of 5000 mg/kg bw/d. Lower maternal bodyweight gains early in the treatment period were also recorded at the next dose of 500 mg/kg bw/day, but without effects on litter parameters in the same study; however, no maternal toxicity (or developmental effects) were observed up to the maximum dose tested of 1000 mg/kg bw/d in the second rat study. It is to be noted that two different strains of rats were used which could partly explain the difference in maternal toxicity seen in the two studies. Overall, diflufenican does not display a potential to adversely affect the development process in the rat when administered at doses at and far in excess of the limit dose.

In the rabbit there was clear evidence of maternal toxicity at 2500 mg/kg bw/day (pale faeces, red discoloured urine, reduced faecal output associated with reduced food consumption, clear reduction in bodyweight gain) but with no accompanying effects on litter parameters or signs of abnormalities in development. A change in the incidence of the very common skeletal variation rudimentary extra ribs at the high dose level was noted, but nevertheless was within the historical control data provided. Furthermore, the incidence in the concurrent control group was lower than expected. There were no other indications of changed variation or malformation incidences, nor were there any changes in embryo or foetal toxicity measurements. Overall, therefore, there was no evidence of developmental toxicity in rabbits when diflufenican was administered at an excessively high dose (exceeding the limit dose of 1000 mg/kg bw/day) that was associated with clear maternal toxicity.

10.8.6 Comparison with the CLP criteria

The developmental toxicity of diflufenican has been investigated in three OECD guideline-compliant oral studies, two in rats and one in rabbits.

Diflufenican did not show any evidence of developmental toxicity in rats or rabbits even when tested at excessively high doses (well above the limit dose in two studies), at which clear maternal toxicity was noted.

The dossier submitter proposes not to classify diflufenican for developmental toxicity.

Conclusions on classification and labelling:

Not classified – Conclusive but not sufficient for classification

10.8.7 Adverse effects on or via lactation

10.8.8 Short summary and overall relevance of the provided information on effects on or via lactation

There is no substantial evidence from the information available to the dossier submitter that diflufenican causes adverse effects in the offspring via lactation. In a two-generation study in the rat, reduced pup weight gain and reduced litter weights were recorded at ≥ 2500 ppm (equivalent to around 200 mg/kg bw/day) from birth up to day 21. The findings were consistent with the reduced maternal body weight reported during gestation. Cumulative pup loss (day 0-21) was seen at 12500 ppm but the findings were inconsistent between F0 and F1 generations. There were no significant changes at any dose in the attainment of developmental landmarks such as surface righting, startle reflex, air righting, and pupil reflex. The available toxicokinetics studies did not highlight diflufenican at a relevant level in mammary glands or breast milk. Owing to the lipophilic properties of diflufenican, it might be expected that residues could be present in milk. In conclusion, there is no evidence that diflufenican affects offspring through an effect on or via lactation. The findings in reduced pup weight gain observed during the post-natal period are most likely to be associated with reduced body weight at birth resulting in a reduced ability to thrive. Therefore the dossier submitter does not propose classification of diflufenican for effects on or via lactation.

Conclusions on classification and labelling:

Not classified – Conclusive but not sufficient for classification

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

The DS included one GLP and OECD compliant two-generation study in the rat to assess the effects of diflufenican on sexual function and fertility. Diflufenican was administered in the diet at concentrations of 0, 500, 2 500 and 12 500 ppm (the corresponding doses in mg/kg bw/day are presented in the table "Mean achieved intakes of diflufenican for each generation", in the section "Assessment and comparison with the classification criteria", below). The DS noted that the top dose approached or exceeded the limit dose (1 000 mg/kg bw/d) for the study protocol.

Both males and females showed an overall reduction in food consumption and body weight gain in the mid and high dose groups. In the high dose group, an increased incidence of mortality was observed in females just prior to parturition and until post-partum day 8. This dystocia-related effect was considered by the DS to be treatment related. No other effects on fertility parameters were observed. However, other findings included a decrease in relative thymus weight in the high dose group which was associated with a depletion of thymic cortical tissue. Further, an increase in liver weight (adjusted for body weight) and

some renal histopathology changes were observed in the high dose group. No relevant microscopic findings were found in the reproductive tract and no notable effects were associated with the endocrine system.

The dystocia was observed as perinatal death of several dams with no or incomplete delivery of their litters. In the high dose group, the incidence was 5.1 % (5/98 pregnant animals) spread across 2 generations and 3 matings (F0B, F1A and F1B). In addition, a single incidence was observed in the mid dose group in the F0 generation. The available historical control data indicate an incidence of dystocia up to 0.5 %, however in general dystocia is regarded as a rare event in rats.

Maternal toxicity was observed as reduced body weight of F0 dams by 11-17 % and for F1 dams by 10-18 % in the high dose group. No other clinical signs were observed. The individual body weight data for dams that died were not available to the DS, so the maternal toxicity of the affected dams could not be assessed on an individual basis.

As regards litter findings, a slight dose-dependent decrease in mean pup weight was observed at birth and during pup rearing. In the high dose group, the treatment was associated with a marked reduction in the ability of litters to thrive up to weaning. The most consistent effect was on litter and mean pup weight, and on some occasions increased pup deaths. The decreased birth-weight of pups occurred only in conjunction with decreased maternal body weights. The DS therefore considered this effect to be secondary to maternal toxicity. No adverse effects were observed on sex ratios, the stages of pre-weaning development or the incidences of anomalous pups. A reduction of mean thymus or spleen weight and an increase in liver-weight were observed in the mid and high dose groups in some batches of weanlings in the parental generations. No effects on the reproductive tract were observed.

The DS included several short-term and long-term studies in the rat, mouse and dog as additional information on possible effects of diflufenican on reproductive organs and tissues. In most of these studies the ovaries, testes and pituitary gland were collected and weighed. In some studies, the uterus and cervix were also analysed for weight changes. None of the studies reported any findings on the reproductive organs analysed. The DS considered these studies as supportive/additional information for the evaluation of effects on sexual function and fertility.

The DS concluded that no classification is warranted for effects of diflufenican on sexual function and fertility. Although the incidence of dystocia seemed to be related to exposure to diflufenican, the finding was observed only at high doses (above the limit dose), together with maternal toxicity and was most likely a secondary, non-specific consequence of other toxic effects. This was further supported by the lack of evidence that diflufenican affects the reproductive tract, the endocrine system or other systems that might be involved in parturition in rats.

Adverse effects on development

Three developmental studies (OECD TG 414) were available to the DS, two in the rat and one in the rabbit. In the two studies with the rat, diflufenican was administered by oral gavage at doses up to 1 000 and 5 000 mg/kg bw/day respectively. In one study, clear signs of maternal toxicity were observed at 5 000 mg/kg bw/day. Litter size and litter weight were also slightly lower at this high dose level. This was possibly linked to maternal

toxicity. However, no clear signs of developmental toxicity were observed even at the excessively high dose level of 5 000 mg/kg bw/day. In the same study, a reduction in maternal body weight gain were observed early in the treatment period at 500 mg/kg bw/day, however no effect on litter parameters were observed at this dose level. In the other study, no maternal toxicity or developmental effects were observed up to the highest tested dose of 1 000 mg/kg bw/day. Two different strains of rats were used in these two studies, which could explain the difference in maternal toxicity observed.

In the rabbit study, clear evidence of maternal toxicity such as pale faeces, red discoloration of the urine, reduced faecal output, reduced food consumption and clear reduction in body weight gain were observed at 2 500 mg/kg bw/day. However, no effects on litter parameters or other developmental effects were observed. It should be noted that the incidence of rudimentary extra ribs at the high dose was noted but was within the range of the historical control data (HCD) provided. Furthermore, the incidence in the concurrent control group was lower than expected.

Based on these three studies, the DS concluded that diflufenican did not show evidence of developmental toxicity in rats or rabbits although tested at doses well above the limit dose and showing clear maternal toxicity in two studies. No classification for developmental toxicity was proposed by the DS.

Effects on or via lactation

The DS concluded that no classification is warranted for effects on or via lactation. This is based on the absence of any substantial evidence from the information available to the DS indicating that diflufenican causes adverse effects in the offspring via lactation.

Comments received during public consultation

Comments were received from two MSCA and one Company-Manufacturer.

One MSCA supported the proposal that no classification is warranted for reproductive toxicity for diflufenican. Another MSCA however questioned the conclusion for no classification for fertility and suggested that a classification in category 2 for effects on sexual function and fertility based on the incidences of dystocia. This classification could be based on the incidence of dystocia being clearly above the HCD and that the top dose only slightly exceeded the limit dose. Further, they questioned the relevance of maternal toxicity for dystocia and considered that the relevance of potential bioaccumulation should be assessed.

The Company-Manufacturer supported no classification for reproductive toxicity as proposed by the DS. They considered the dystocia observed to be secondary to the excessive general toxicity of diflufenican.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The DS included one 2-generation study (conducted according to GLP and OECD TG 415, 416) in Sprague Dawley rats for the assessment of effects on sexual function and fertility (Anon. 1985). In addition, histopathology of the reproductive tract in F0 and F1A adult rats

were examined in Anon. (1987). Diflufenican was administered in the diet at dose levels of 0, 500, 2 500 and 12 500 ppm. The mean achieved intakes of diflufenican are summarised in the table below. It is noted that the high dose group approached or even exceeded the limit dose.

Table: *Mean achieved intakes of diflufenican for each generation.*

Generation/phase	**Mean diflufenican intake (mg/kg bw/day)**					
	Males			**Females**		
	500 ppm	**2 500 ppm**	**12 500 ppm**	**500 ppm**	**2 500 ppm**	**12 500 ppm**
F0/premating	35.5	175.6	888.0	41.9	206.1	1 042.0
F1A/premating	38.8	198.4	1 035.0	47.3	223.7	1 168.0
F2A/rearing	42.9	209.8	1 044.0	50.8	244.2	1 316.0

F0 animals (32/sex/group), 6 weeks of age, were treated for 10 weeks pre-mating until sacrifice after weaning of F1B pups. F1A animals (28/sex/group), 4 weeks of age, were treated for 12 weeks during the pre-mating period until sacrifice after weaning of the F2B pups. F2A animals were treated from 4 weeks of age for 90 days before sacrifice while F2B animals were treated from 4 weeks of age for 14 days before sacrifice.

Findings of parental toxicity are summarised in the table below.

Table: *Summary of parental toxicity*

F0 generation			
	500 ppm	**2 500 ppm**	**12 500 ppm**
Perinatal mortality	1 female on day 18 of the first mating period. Not pregnant.	1 female, second mating at GD 22	Second mating: 1 female PND8 (humane sacrifice), 1 female GD 22.
Food consumption, bw and bw gain	No effect observed	Males/females: reduced food consumption and bw gain (~10 % compared to controls).	Males/females: reduced food consumption (~10-15 % compared to controls) and bw gain (~15 % compared to controls).
Organ findings	Significantly reduced relative thymus weight*, dose-dependent ranging from 8 % at 500 ppm to 15 % at 12 500 ppm		
Histopathology of the reproductive tract	No adverse effects observed in males or females (only investigated in the control and high dose groups)		
F1 generation			
	500 ppm	**2 500 ppm**	**12 500 ppm**
Perinatal mortality	1 female (first mating)	None	First mating: 1 female PND3 (humane sacrifice), 1 female PND8. Second mating: 2 females PND1
Food consumption, bw and bw gain	No effect observed	Males/females: reduced food consumption (~10-20 % compared to controls) and bw gain (~15% compared to controls).	Males/females: reduced food consumption (~10-20 % compared to controls) and bw gain (~10-15 % compared to controls).
Organ findings	No adverse effects observed	Males: 15.5 % reduced thymus weight (relative to brain) compared to control.	F1A males: 7 %* increased relative (to bw) kidney weight compared to controls 25 % reduced relative (to brain)

			thymus weight compared to controls; F1A females: 11.5 % reduced relative (to brain) thymus weight compared to controls.
Histopathology (lungs, thymus, liver, spleen, kidneys and reproductive tract)	No treatment related effects observed	Dilated renal medullary collecting ducts (1/28 females).	Dilated renal medullary collecting ducts (2/24 females), associated with mineral casts in one of the affected females. Minimal depletion of cortical tissue in thymus in 4/28 males and 6/24 females, associated with lower relative thymus weights in both sexes. No adverse effects on reproductive tract (males/females).

* p < 0.05, ** p < 0.01, *** p < 0.001 (Kruskal-Wallis test intergroup comparison with the control)

An overall reduction in food consumption was observed in both males and females at 2 500 and 12 500 ppm (~10-15 % compared to controls). In F0 adult animals food consumption decreased during the pre-mating phase in males and females in the mid and high dose. In the F1 and F2 males a similar decrease in food consumption was seen in all treated groups. For F1 and F2 females there was a decrease in food consumption of 10-20 % and 5-10 % in the mid and high dose groups respectively. Body weight gain was also reduced (10-20 %) in both males and females in all generations in the mid and high dose groups, however, similar findings were not observed for the corrected bodyweight gain (see the table below).

Table: *Corrected body weight gain (g) GD 0-20*

	Litter	**0 ppm**	**500 ppm**	**2 500 ppm**	**12 500 ppm**
F0	F1A	42.7 g	39.4 g	43.3 g	49.7 g
	F1B	48.1 g	47.6 g	53.6 g	47.8 g
F1	F2A	41 g	41.2 g	46.6 g	47.1 g
	F2B	43.3 g	42.2 g	42.8 g	47.5 g

Mating performance and pregnancy rate were unaffected in all pairings. No effects on the reproductive tract of females in the control and high dose groups were revealed in the histopathological examination (Anon. 1987). No treatment-related effects were observed in the males; over the two generations, the number of males which failed to induce pregnancy at either mating was 1, 2, 3 and 1 at 0, 500, 2 500 and 12 500 ppm, respectively. Two males in the mid dose group showed reduced spermatogenesis with reduced numbers of spermatozoa in the epididymides. For all other animals failing to mate, no histopathological findings of the reproductive tract outside normal limits were reported.

As regards mortalities, nine females died or were killed prematurely during the study. Two of these were clearly incidental, whilst up to 6 of the 7 remaining female deaths were plausibly linked to dystocia. The female deaths are further described in the table below. In addition, two males died during the study (one F1A generation control male in week 30, one F0 generation male at 500 ppm in week 29) but these were not considered treatment related.

The gestation period was unaffected by treatment in all four pairings. Five out of 7 dams found dead at or near parturition were found to have died during delivery of the pups resulting from the second mating of the F0 or F1 adults, and 6 of the 7 animals were in the high dose group (1 042/1 168 mg/kg bw/day in F0/F1 females).

In the high dose group, 4 out of 6 dams that died had failed to deliver all their offspring and were therefore clearly diagnosed with dystocia. Of the remaining two females that died, one female (second mating) was humanely sacrificed at PND8 due to poor condition linked to paralysis of the hind-limbs. A distended uterus was observed at autopsy. No clear information was available regarding whether the paralysis occurred before, during or after parturition. The paralysis could be linked to difficult parturition and dystocia cannot be ruled out. The other female displayed no evidence of difficult parturition.

A single incidence of dystocia (incomplete parturition) occurred at the mid-dose level in the F0 generation but no evidence of dystocia was seen at this dose in the F1 generation.

Table: *Incidence of perinatal mortality and potential dystocia in F0 and F1 dams*

Dose level	Dam ID	Time/cause of death/clinical sign	Dystocia
Control	-		
500 ppm	F0A 172	Humanely sacrificed day 18 of first mating period. Not pregnant. Death caused by poor clinical condition and dorsal injury.	No
	F1A 422	Right eye prominent and congested. Autopsy – right eye ruptures and haemorrhagic. Left lower molar, crown missing.	No
2 500 ppm	F0B 221	Found dead day 22 of second gestation period. All 15 foetuses undelivered.	Dystocia plausible.
12 500 ppm	F0B 235	Humanely sacrificed on PND8. Paralysis of hind limbs. No delayed gestation, no undelivered foetuses. All pups died by PND7, likely due to starvation.	Dystocia cannot be dismissed.
	F0B 237	Found dead on day 22 of the second gestation. All 14 foetuses undelivered.	Dystocia plausible.
	F1A 479	Found dead PND8 of first litter. No undelivered foetuses, though only 2 born. No increase in the duration of gestation.	Not dystocia.
	F1A 459	Humanely sacrificed PND3. Duration of parturition extended. 6 foetuses undelivered. 2 foetuses undelivered at necropsy. Other findings: pallor, piloerection, blood staining of the fur around vaginal opening.	Dystocia plausible.
	F1B 474	Found dead PND1 of second mating. Parturition incomplete, 3 foetuses delivered, 12 undelivered. No increase in duration of gestation.	Dystocia plausible.
	F1B 478	Found dead on PND1 of second mating. Parturition incomplete. 1 pup born, 13 undelivered. No increase in duration of gestation.	Dystocia plausible.

Regarding historical control data for dystocia in rats, data collected from 3 preliminary one-generation studies and 7 definitive two-generation studies from the same laboratory, in the same strain of animals from 1981-1988 were compiled by the study sponsor. Dystocia was identified in control animals in 2/10 studies with one incidence in a study from 1981 and two incidences in a study from 1983. The animal supplier indicated the incidence of perinatal mortality in their breeding colonies of approximately 0.2-0.5 %.

In comparison, the incidences of assumed dystocia identified in the two-generation study with diflufenican are summarised in the table below. It should be noted that there was no overall effect of diflufenican on the duration of gestation in any group.

Table: *Incidence of diagnosed dystocia in F0 and F1 dams in the study.*

	Dystocia/litters/mated female rats			
Mean diflufenican intake	**0 ppm**	**500 ppm**	**2 500 ppm**	**12 500 ppm**
F0 first mating % incidence of dystocia	0/32/32 0	0/30/32 0	0/31/32 0	0/29/32 0
F0 second mating % incidence of dystocia	0/25/32 0	0/21/31 0	1/23/32 4.3	2/23/32 8.7
F1 first mating % incidence of dystocia	0/26/28 0	0/25/28 0	0/24/28 0	1/24/28 4.2
F1 second mating % incidence of dystocia	0/26/28 0	0/26/28 0	0/26/28 0	2/22/26 9.1
Total number (%) incidence of dystocia in pregnant rats across matings/ dose a) group	0/109/120 (0)	0/102/120 (0)	1/104/120 (1)	5/98/120 (5.1)

Findings of litter toxicity are summarised in the table below.

Table: *Summary of litter toxicity*

	F1 pups (excluding total litter loss)			
		500 ppm	**2 500 ppm**	**12 500 ppm**
	Pup weight			
		No effect observed	F1A ↓** & F1B ↓*** litter and mean pup weight day 21.	F1A ↓** & F1B ↓*** litter and mean pup weight day 21. F1A ↑** cumulative pup loss/pup mortality (day 0-21).
		Dose dependent decrease in mean pup-weight observed in F1A and F1B from birth up to PND21 (weaning)		
	Organ findings (relative organ weight adjusted to bodyweight)			
F1A young		Females ↓* spleen weight (10 % reduction compared to controls)	Females ↓** spleen weight (11% reduction compared to controls); males ↓* spleen weight (10 % reduction compared to controls).	Females ↓** spleen weight (14 % reduction compared to controls) ↓* thymus weight (17 % reduction compared to controls); males ↓* spleen weight (13 % reduction compared to controls).
		Note: Thymus weight change was dose-dependent in both sexes.		
F1A adults		No effect observed	No effect observed	Males ↑* kidney weight (7 % increase compared to controls).
	F2 pups (excluding total litter loss)			
		500 ppm	**2 500 ppm**	**12 500 ppm**
	Pup weight			
		No adverse effects observed	F2A & F2B ↓* mean pup weight day 21	F2A ↓** litter weight ↓*** mean pup weight day 21; F2B ↓*** litter weight, ↓* mean pup weight day 21, ↑** cumulative pup loss/pup mortality (day 0-21).
		Dose-dependent decrease in mean pup weight observed at F2A & F2B from birth up to post-natal day 21 (weaning)		
	Organ findings			
F2A young		No adverse effect observed	Male ↓** spleen weight (15 % reduction	Male ↑** liver weight (10 % increase compared to controls) ↓** spleen weight

			compared to controls)	(12 % reduction compared to controls)
F2 adult		Males ↑** kidney weight (9.3 % increase compared to controls); females ↑** liver weight (12% increase compared to controls).	Males ↑* kidney weight (11 % increase compared to controls); females ↑** liver weight (14 % increase compared to controls).	Males ↑* kidney weight (8.4 % increase compared to controls); females ↑** liver weight (20 % increase compared to controls). Increase in liver weight was dose-dependent.
F2B young		500 ppm: no adverse effect observed	Males ↑** liver weight (9 % increase compared to controls).	↑** liver weight (9 % increase compared to controls).
	Histopathology (lungs, thymus, liver, spleen, kidneys) F2A weanlings			
		No treatment-related effects observed	Dilated renal medullary collecting ducts in 2/24 females associated with mineral casts in the dilated collecting ducts of one female.	Dilated renal medullary collecting ducts in 1/19 males and 2/17 females associated with mineral casts in all affected weanlings. Wedge-shaped areas of dilated cortical tubules were also observed in 2/19 male weanlings and 1/17 female weanlings.

* p < 0.05, ** p < 0.01, *** p < 0.001 (Kruskal-Wallis test intergroup comparison with the control)

A generally low incidence of total litter loss was reported. Overall the incidence was 0, 0, 0, 0 (F0, 1st mating) and 1, 0, 0, 1 (F0, 2nd mating) in control, low, mid and high dose group and 1, 4, 0, 3 (F1A, 1st mating) and 0, 0, 1, 1 (F1A, 2nd mating) in the control, low, mid and high dose group, respectively. Mean pup weights at birth were decreased in a dose-dependent manner and were statistically significantly different for all matings in the high dose groups. This was also generally the case but to a lesser extent in the mid dose groups. The reduced pup weight was associated with a statistically significant decrease in mean litter weight in 2/4 matings, while litter size at birth was unaffected.

Pup weight gain from birth up to day 21 was statistically significantly lower in the high dose group, and to some degree also in the mid-dose group, in both generations. In the high dose groups a statistically significant increase in pup mortality (cumulative pup loss between days 0-21) at the first mating of the F0 generation and second mating of the F1A generation were noted. However, no clear dose-response relationship was seen for the cumulative pup loss at day 21 (across 4 matings) and they were statistically significantly different from control only in 2/4 matings in the high dose groups.

Overall, the litter effects observed in the high dose group are likely to be the consequence of the markedly lower pup weight observed at birth compared to controls, resulting in a marked reduction in the ability of litters to thrive up to weaning (day 21). Maternal weight was affected in a dose-dependent manner during gestation, however no effect was observed for the corrected maternal body weight gain. Despite their initially reduced bodyweight at birth, the surviving pups in the high dose group gained weight in a similar pattern to the other dose groups up to weaning.

No treatment-related effects were seen on sex ratio or pre-weaning development. The incidence of structural anomalies recorded at autopsy of excess F1 and F2 offspring did not indicate any adverse relationship to dietary concentration of diflufenican.

Table: *Litter data up to day 21*

Dose (ppm)	**0**	**500**	**2 500**	**12 500**
F1 pups 1st mating				
Litter size at birth	11.8	12.0	12.0	11.9
Mean pup weight at birth (g)	6.0	5.9	5.7*	5.2***

Cumulative pup loss (day 0-21) %	3.4	5.6	6.4	11.2**
Mean litter weight (g)	504.6	497.3	444.7***	408.0***
Mean pup weight day 21 (g)	43.8	42.7	38.0***	34.8***
F1 pups 2nd mating				
Litter size at birth	10.7	11.8	10.2	10.3
Mean pup weight at birth (g)	6.1	5.9	5.8*	5.7**
Cumulative pup loss (day 0-21) %	11.8	8.4	15.1	7.9
Mean litter weight day 21 (g)	486.1	497.2	395.8**	377.3***
Mean pup weight day 21 (g)	45.8	43.7	40.9**	38.4***
F2 pups 1st mating				
Litter size at birth	11.0	11.7	12.0	10.5
Mean pup weight at birth (g)	5.8	5.8	5.6	5.4**
Cumulative pup loss (day 0-21) %	7.3	5.7	4.9	13.0
Mean litter weight (g)	459.7	482.1	455.5	369.8**
Mean pup weight day 21 (g)	43.3	42.4	39.0*	36.0***
F2 pups 2nd mating				
Litter size at birth	12.4	12.3	12.4	9.5**
Mean pup weight at birth (g)	5.9	5.8	5.8	5.4**
Cumulative pup loss (day 0-21) %	6.4	9.2	4.2	26.4**
Mean litter weight day 21 (g)	480.7	484.4	449.6	329.3***
Mean pup weight day 21 (g)	39.3	40.4	36.5*	35.3*

* p<0.05, ** p<0.01, *** p<0.001 (Kruskal-Wallis test intergroup comparison with the control)

In F1A weanlings a decrease in thymus weight was observed in the high dose group. Male F1A and F2A weanlings showed a reduction in spleen weights in the mid and high dose groups, and female F1A weanlings showed a reduction of spleen weights in all dose groups. No histopathology findings were associated with these organ weight changes.

Increased liver weights were observed in young males in the F2A and F2B generations at the higher doses (less than 10 % increase compared to controls). In adult F2A female animals a significant increase in liver weight was seen in all treatment groups, however the control value appeared to be low in this group (10-20 % increase compared to controls). No microscopic changes in the liver were observed.

Kidney changes were apparent in the F2A weanlings which were considered to be treatment related, including dilated collecting ducts in 2/17 females and 1/19 males in the high dose groups; this was associated with mineral casts in all weanlings. Wedge-shaped areas of dilated cortical tubules were seen in 2/19 male weanlings and 1/17 female weanlings. In the mid dose group, dilated medullary collecting ducts were observed in 2/24 weanling females.

RAC noted the repeated dose toxicity studies included by the DS (summarised under "Supplemental information" in the background document; for further detail see Section 10.10 of the CLH report). Overall, these studies did not show any evidence on effects on reproductive organs.

Comparison with the CLP criteria

No human data was available, therefore, classification as Repr. 1A is not warranted.

In a GLP and OECD TG compliant two-generation study in the rat, diflufenican was administered via the diet up to a high dose level which were up to or above the limit dose. Dystocia were observed in one dam of the mid dose groups and 5 dams in the high dose groups. The dystocia was observed together with some maternal toxicity observed as reduced food consumption and reduced body weight gain. It is noted, however, that no clear effect on corrected maternal body weight gain on GD 0-20 was observed.

RAC is of the opinion that classification in Category 1B is not justified since the findings of dystocia do not provide sufficiently clear evidence of an adverse effect on sexual function and fertility.

As regards a classification in category 2 or no classification, RAC considers the cases of dystocia to be related to the exposure to diflufenican. Five animals in the high-dose group were affected across generations and matings. In addition, one single incidence was observed in the mid-dose group in the F0 generation. The incidence in the high dose group was clearly above the HCD provided. However, it is noted that the high dose group exceeded the limit dose. Maternal toxicity was evident at this dose as body-weights of the dams were up to 17% lower than the control animals throughout the study. However, no clear effect was seen on the corrected maternal body weight gain (GD 0-20). There is no evidence from the reproductive toxicity or repeated-dose toxicity studies indication that diflufenican acts through a specific mode-of-action that might result in dystocia. No other effects on reproduction or fertility were observed in this study.

In conclusion, RAC is of the opinion that the findings in this study provide some evidence of an effect on sexual function or fertility. However, other toxic effects observed in the dams and the fact that the effect is mainly observed at a dose level exceeding the limit dose decrease the concern. Therefore, RAC is of the opinion that **no classification** is justified for adverse effects on sexual function and fertility.

Adverse effects on development

Three developmental toxicity studies were available for the evaluation of adverse effects on development, two studies in the rat and one study in the rabbit. These studies have been summarised in table below.

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

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**
Oral gavage OECD TG 414 (1981) Sprague Dawley rats Pregnant	Diflufenican (purity 98.1 %) 0, 50, 500 and 5 000 mg/kg bw/day Administered by gavage at	Maternal toxicity: Dose-related ↑salivation (post-dosing); not seen beyond the last dosing day. Pale faeces in high dose animals over days 7 to 16. No treatment-related deaths. ≥ 500 mg/kg bw/day: dose-related ↓bodyweight gain throughout treatment.

females 25 / group Anon. 1984a	dose volume of 2 mL/100g bw Treated between days 6 and 15 of pregnancy	Food consumption at 5 000 mg/kg bw/day slightly lower than controls (~10 % reduction) (days 6-10). No gross necropsy findings. Developmental toxicity: No statistically significant differences in litter size, litter weight or pre- or post-implantation losses between the treated and the control groups. Sex ratio unaffected by treatment. No dose-related increase and no pattern to the malformations observed. Dose-dependent mean increase (%) in **visceral anomalies** observed although no obvious pattern or relationship between the anomalies seen. No historical control data available. No treatment-related increase in skeletal anomalies observed.
Oral gavage OECD TG 414 (1981) Wistar rats Pregnant females 28/group Anon. 2002	Diflufenican (purity 98.8 %) 0, 250, 500 and 1 000 mg/kg bw/day Administered by gavage at dose volume of 5 mL/kg bw Treated between days 6 and 15 of pregnancy	Maternal toxicity: No clinical signs. No treatment-related deaths. Food consumption / bodyweight gain comparable to the control group at all doses. Comparable mean number of corpora lutea, implantations, early and late resorptions, pre- and post-implantation loss and dams with any/all resorptions to the respective vehicle control values in all treatment groups. No gross necropsy findings Developmental toxicity: No statistically significant differences in litter size, litter weight between the treated and the control groups. Sex ratio unaffected by treatment. No treatment-related external observations. No visceral observations or major skeletal malformations.
Oral gavage OECD TG 414 (1981) New Zealand White rabbits Pregnant females Anon. 1984b	Diflufenican (purity 98.1 %) 0, 50, 350 and 2 500 mg/kg bw/day Administered at dose volume of 10 mL/kg bw Treated between days 6 and 18 of pregnancy	Maternal toxicity: No treatment-related deaths. 2500 mg/kg bw/day: pale faeces (up to day 19), red discoloured urine (11/16 animals towards end of treatment, persisting few days post dosing period), reduced faecal output associated with reduced food consumption. 350 mg/kg bw/day: red discoloured urine (3/13 animals) at immediate post-dosing period. 2 500 mg/kg bw/day: food consumption and bodyweight gain clearly reduced throughout the treatment period but recovered once treatment ceased. 50-350 mg/kg bw/day: food consumption lower than controls, however not considered treatment related because the reduction was observed during the pre-treatment period and remained as such throughout treatment. One dam at 350 mg/kg bw/day aborted its litter on day 24 (10 abortion sites in the uterus). No treatment-related increase in post-implantation losses and litter size. No treatment-related gross necropsy findings Developmental toxicity: No treatment-related effect on litter weight, mean foetal weight or sex ratio. No treatment related skeletal observations. Variant sternebrae incidence not increased by treatment.

		↑ Extra ribs incidence in all treated groups in a dose-related pattern (statistically significant at 2 500 mg/kg bw/day), however within historical control data range provided. Incidence in control animals relatively low.

In a study conducted according to OECD TG 414 and GLP, rats (Sprague Dawley, 25/group) were exposed to diflufenican (98.1%) at dose levels of 0, 50, 500 and 5 000 mg/kg bw/day (Anon., 1984a). A dose-related increase in salivation were observed in the in the post-dosing period, accompanied by brown facial staining on many occasions. The increased salivation was not observed beyond the last day of dosing. In the high dose animals, pale faeces were also reported over GD 7 to 16. Food consumption was slightly reduced (~10 %) in the high dose group over GD 6 to 10. Bodyweight gains were reduced in a dose-related pattern in the mid and high dose groups, beginning from day 6 and persisting throughout the treatment period (to GD 15). However, these effects showed at least partial recovery after the cessation of treatment. There were no gross necropsy findings in dams. No statistically significant effects on litter size, litter weight or pre- or post-implantation loss were observed and sex ratio was not affected by treatment. There was no dose-related increase and no pattern to the malformations observed. Skeletal anomalies were not increased. The number of young with visceral anomalies was increased in all treated groups in a dose-related pattern (3.2 %, 4.7 %, 5.8 % and 9.6 % at 0, 50, 500 and 5 000 mg/kg bw/day respectively, see the table below), however there was no obvious pattern or relationship between the anomalies seen. No HCD were available to put this finding into context. The incidence of foetuses with additional ribs or sternebral abnormalities was not affected by treatment.

Table: *Group mean incidence of malformations and anomalies*

Group (mg/kg bw/day)				**0**	**50**	**500**	**5 000**
# litters				19	21	19	21
# pups with	**Malformations**		Examined	199	205	195	207
			Total (N)	3	5	1	3
			Mean (%)	1.5	2.6	0.7	1.4
	Anomalies	**Skeletal**	Examined	98	101	97	102
			Total (N)	13	16	12	14
			Mean (%)	14.3	16.1	13.9	13.3
		Visceral (Wilson technique)	Examined	98	99	97	102
			Total (N)	3	5	5	10
			Mean (%)	3.2	4.7	5.8	9.6
			# litters affected	2	5	5	7

In another study according to OECD TG 414 and GLP, rats (Wistar, 28/group) were exposed to diflufenican (98.8 %) by oral gavage at doses of 0, 250, 500 and 1 000 mg/kg bw/day (Anon. 2002). No evidence of developmental toxicity in rats were observed. There were no clinical signs or treatment-related deaths observed, no effects on body weight and body weight gain or food consumption. There were no effects on the mean number of corpora lutea, implantations, early and late resorptions, pre- and post-implantation loss and dams with resorptions. There were no treatment-related gross visceral lesions in the rats sacrificed at term. Litter parameters such as mean litter size, number and weight of the foetuses and sex ratio were statistically comparable between exposed and control

groups. No treatment-related changes in external observations (including major malformations), skeletal variant parameters, visceral malformations or anomalies were observed in exposed groups compared to the control group.

In a study conducted according to OECD TG 414 and GLP, New Zealand White rabbits were exposed to diflufenican (98.1%, oral gavage) at doses of 0, 50, 350 and 2 500 mg/kg bw/day (Anon., 1984b). There were no treatment-related deaths. In the high dose group, clinical signs included pale faeces (for most animals during most of the treatment period, persisting as far as GD 19) and red discoloured urine (in 11 out of 16 animals towards the end of the treatment period, persisting a few days into the post-dosing period). In addition, reduced faecal output was observed. This was associated with reduced food consumption, which was clearly reduced at 2 500 mg/kg bw/day throughout the treatment period but recovered once treatment ceased. Bodyweight gain was markedly lower in the high dose group compared to controls during the treatment period, especially early in the treatment period. This effect recovered once treatment ceased. A single animal in the mid dose group aborted on GD 24. There was no treatment related gross necropsy findings in dams and no treatment-related effect on post-implantation losses, litter size, litter weight, mean foetal weight or sex ratio. One or 2 incidences of malformations were observed in each dose group. The pattern of anomalies identified by gross dissection or skeletal examination did not indicate any effect of treatment. The incidence of variant sternebrae was not affected by treatment. The incidence of the common variation rudimentary extra ribs was higher than controls in all treated groups, and statistically significant in the high-dose group (see table below). However, the incidences were within the historical control range.

Table: *Incidence of extra ribs and sternebrae variants*

Dose (mg/kg bw/day)	**N**	**Foetuses examined**	**12 ribs**		**13 ribs**		**Normal sternebrae**		**Variant sternebrae**	
			Total	Mean %	Total	Mean %	Total	Mean %	Total	Mean %
0	12	96	80	81.2	16	19.0	66	71.7	30	28.3
50	16	111	74	63.6	37	36.4	89	85.6	22	14.4
350	13	97	65	60.9	32	39.1	73	78.0	24	22.0
2 500	16	124	73	57.8	51	**42.2***	114	92.5	10	7.5

* $p < 0.05$

HCD for extra ribs from 21 rabbit teratology studies from the same laboratory (Jan. 1983 – Jan. 1984) showed a mean incidence of 34.3 %, ranging from 13.8-50 %.

Comparison with the CLP criteria

In one of the two rat developmental toxicity studies, the highest dose of diflufenican greatly exceeded the limit dose. Maternal toxicity was observed in the high dose group, including pale faeces, reduced body weight gain and food consumption. Litter size and litter weight were also slightly lower. The incidence of visceral anomalies was increased in all treated groups in a dose-related pattern (3.2 %, 4.7 %, 5.8 % and 9.6 % at 0, 50, 500 and 5 000 mg/kg bw/day respectively), however there was no obvious pattern or relationship between the anomalies seen and no HCD were available. In the other rat developmental toxicity study, no maternal toxicity or developmental effects were observed up to the maximum dose tested of 1 000 mg/kg bw/day. In the rabbit, there was clear evidence of maternal toxicity at the high dose of 2 500 mg/kg bw/day. No effects on litter

parameters or signs of abnormalities in development were observed.

In conclusion, based on the available studies, RAC agrees with the DS assessment that no classification for effects on development is warranted.

Effects on or via lactation

There was no human evidence indicating a hazard to babies during the lactation period. A two-generation study in rats showed reduced pup weight gain and reduced litter weights in the mid and high dose group from birth up to post-natal day 21. This was consistent with the reduced maternal body weight reported during gestation. In the high dose group, a cumulative pup loss (day 0-21) was observed, however the findings were not consistent between F0 and F1 generations. No significant changes at any dose in effects such as surface righting, startle reflex, air righting, and pupil reflex were observed. Diflufenican has lipophilic properties, which indicates that residues could be present in milk. Toxicokinetic studies did not indicate that diflufenican was present at a relevant level in mammary glands or breast milk.

In conclusion, there is no evidence that diflufenican affects offspring through an effect on or via lactation. The reduced pup weight gain observed during the post-natal period could be associated with reduced body weight at birth rather than a result of effects on or via lactation. RAC is therefore of the opinion that no classification for effects on or via lactation is justified.

Overall, RAC is of the opinion, in line with the DS that **diflufenican does not warrant classification for reproductive toxicity**.

Supplemental information - In depth analyses by RAC

Supportive studies

The DS included several repeated dose studies by the oral route as supporting information on possible effects of diflufenican on reproductive organs and tissues. These included six studies in the rat, one study in the mouse and two studies in the dog. No information is available for dermal and inhalation toxicity. In addition, two combined oncogenicity and repeated toxicity studies in rats and mice were also conducted for a period of 104 weeks. In most of the studies the ovaries, testes and pituitary gland were collected and weighed. More occasionally, the uterus and cervix were analysed for weight changes. None of the studies reported any findings on the reproductive organs analysed.

In a 2-week study with Sprague Dawley rats (5/sex/dose, doses up to 1 600 mg/kg bw, oral gavage daily) a dose-dependent increase in bilirubin in males was noted at doses greater than 800 mg/kg bw/day. Reproductive organ weight data and histopathological findings did not suggest any treatment-related effect on the reproductive system (Anon. 1983).

In a 28-day range-finding study with Wistar rats (6/sex/dose, doses up to 674.2/668.7 mg/kg bw/day, diet) food consumption was significantly reduced from 27.2 mg/kg bw/day in females. Body weight gain was significantly reduced from 134 mg/kg bw/day. No other relevant findings were noted such as for the reproductive organs analysed in this study at

any dose level (Anon. 2001).

In a 13-week study with Sprague Dawley rats (15/sex/dose, doses up to 3 448-3 726 mg/kg bw/day, diet, 13 weeks), statistically significant reductions in bodyweights were observed from 37.5 mg/kg bw/day in males. However, the validity of the study is questioned due to findings of chronic respiratory disease in the majority of animals in control and top dose groups (Anon. 1984c).

In a 13-week study with Sprague Dawley rats (15/sex/dose, doses up to 38.1-44.3 mg/kg bw/day, diet) alopecia was evident in 5/15 females at 44.3 mg/kg bw/day. Food consumption and body weight gain were reduced from 8.7 mg/kg bw/day in females for most of the duration of the study. Bodyweight gain was statistically significantly reduced in males in the high dose group towards the latter part of the study. No adverse effects were noted for the reproductive organs analysed in this study (Anon. 1985a).

In a 13-week study with F344 rats (35/sex/dose, doses up to 196.6 mg/kg bw/day, diet, recovery groups 4 and 8 weeks), bodyweight gains were statistically significantly reduced in males/females from 19.47 mg/kg bw/day, however this effect was reversible. At 196.6 mg/kg bw/day a statistically significant change in relative liver weight was observed in males/females (reversible), as well as a slightly increased incidence of hepatic hypertrophy in males (reversible). No other relevant findings were noted such as for the reproductive organs at any dose level (Anon. 1987b)

In a 90-day study with Wistar rats (10/sex/dose, doses up to 280.4/312 mg/kg bw/day, diet, 90 days, recovery groups for a further 28 days) statistically significantly reductions in food consumption, bodyweight and body weight gain were observed for males/females from 56.7 mg/kg bw/day. Recovery groups showed significant increases in bodyweight gain compared to controls, even though food consumption in females at 312 mg/kg bw/day remained significantly below control levels during the recovery period. No other relevant findings were observed for the reproductive organs analysed (ovaries, uterus, testes and epididymides) at any dose level (Anon. 2002a).

In a 2-year study with F-344 rats (50/sex/dose, doses up to 614-749 mg/kg bw/day, diet) a statistically significantly reduction in body weight gain in females were observed at 23.27-27.78 mg/kg bw/day. However, the reduction was slight compared to control groups (~5 %). At higher doses, body weight gains were significantly reduced in both males and females up to 33 % for females in the high dose group. Liver changes were observed at the top doses. No other relevant findings were observed for the reproductive organs analysed (ovaries, uterus, testes and epididymides) at any dose level (Anon. 1987d).

In a 13-week study with B6C3F1 hybrid mice (10/sex/dose, doses up to 3 598-4 002 mg/kg bw/day, diet) statistically significantly decreased body weight gain in males/females and increased liver weight in males/females were seen from 825.5/1 024 mg/kg bw/day. No notable effects were observed in the reproductive organs investigated in this study at any dose (Anon. 1993).

In a 1986 GLP study with B6C3F1 hybrid mice (52/sex/dose, doses up to 1 618-1 989 mg/kg bw/day, diet, 104 weeks) a statistically significantly reduction in body weight gain were observed from 62.2-73.6 mg/kg bw/day. Liver changes were observed at the mid and high dose. No other relevant findings were observed such as for the reproductive organs analysed (ovaries, uterus, testes and epididymides) at any dose level.

In a 13-week study with beagle dogs (4/sex/dose, doses up to 1 000 mg/kg bw/day, oral gavage) a treatment-related increased incidence of vomiting was noted in all dose groups. A significant decrease in bodyweight gain was seen in females in the mid and high dose groups. In the high dose group, a significant reduction of thymus and lung weight were observed in females. No reproductive organs such as the ovaries and testes were affected (Anon. 1984c).

In a 1-year study with beagle dogs (5/sex/dose, doses up to 1 000 mg/kg bw/day, oral route (capsules)) liver effects were seen from 300 mg/kg bw/day. No reproductive organs such as the ovaries, uterus or testes were affected (Anon 1987c).

10.9 Specific target organ toxicity-single exposure

This endpoint has not been considered in this report.

10.10 Specific target organ toxicity-repeated exposure

Diflufenican has been studied extensively in standard GLP/OECD-compliant studies involving repeated oral treatment of rats and mice for up to 13 weeks, and for up to one year in dogs. No studies were conducted via the inhalation and dermal routes. Further, there are two combined carcinogenicity and toxicity studies in rats and mice, conducted for a period of 104 weeks

In this report, the repeated-dose studies are presented as supporting information for the consideration of the classification of diflufenican for reproductive toxicity.

Table 12: 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**	**CLP guideline value for classification (mg/kg bw/d, rat study)**	**Results**
Oral gavage OECD 407 (1981) GLP CD strain Sprague Dawley rats 5/sex/dose Anon. 1983	Diflufenican Batch no. LOP 4383 Purity not available Oral gavage 0 (vehicle), 400, 800, and 1600 mg/kg bw/day 2 weeks	Cat 1 = 60 Cat 2 = 600	Clinical signs One control animal died overnight after 4 doses. No clinical signs were reported in any treatment group. Haematology / clinical chemistry data ≥ 800 mg/kg bw/day: ↑ bilirubin in males. Ophthalmological/urine/organ weight/ histopathological findings No statistically significant or treatment-related findings observed. NOAEL = 400 mg/kg bw/day LOAEL = 800 mg/kg/day based on dose-dependent increased bilirubin in males. Note: Ovaries (with Fallopian tubes attached), testes and the pituitary gland were analysed for weight change and histopathology.
Oral OECD 407 (1995 GLP Wistar HsdCpd: WU rats 6/sex/dose Anon. 2001	Diflufenican Batch/Receipt No.: 7 Purity 98.8 % Stable in diet for up to 30 days at room temperature Oral, diet 0, 300, 1500 and 7500 ppm (estimated to be 0, 27.5/27.2, 128.9/134 and 674.2/668.7 mg/kg bw/day in males and females) 28 days	Cat 1 = 30 Cat 2 = 300	Clinical signs / deaths None. 27.5 / 27.2 mg/kg bw/d: ↓* food consumption in females, but bodyweight gain unaffected. 128.9 / 134 mg/kg bw/d: ↓* food consumption and bodyweight gain in females. 674.2 / 668.7 mg/kg bw/d: ↓*bodyweight gain in males week 1 only; ↓* food consumption and bodyweight gain in females. NOAEL = males 128.5 mg/kg bw/day; females 27.2 mg/kg bw/day LOAEL = males 674.2 mg/kg bw/day; females 134 mg/kg bw/day based on decreased bodyweight, body weight gain & food consumption. Note: Gonads and epididymides were analysed for weight change and histopathology.
Oral No guideline specified	Diflufenican Batch 12 (purity not reported)	Cat 1 = 10 Cat 2 = 100	Clinical signs / deaths / ophthalmology Single death during blood sampling (not treatment related). 32.8 / 37.5 mg/kg bw/day

Non-GLP CD Sprague Dawley rats 15/sex/dose Anon. 1984c.	Oral, diet 0, 500, 5000 or 50000 ppm (estimated to be 0, 32.8/37.5, 335/383 and 3448/3726 mg/kg bw/day in males and females) 13 weeks		↓* bodyweight in males only. <u>335 / 383 mg/kg bw/day</u> ↓** bodyweight and ↓ bodyweight gain both sexes <u>3448 / 3726 mg/kg bw/day</u> ↓** bodyweight and ↓ bodyweight gain both sexes Note: Majority of controls and high dose groups had evidence of chronic respiratory disease; therefore no NOAEL was set for this study. Note: Ovaries, testes and the pituitary gland were analysed for weight change and histopathology.
Oral Compliant with OECD 408 (1981) Non-GLP CD Sprague Dawley rats 15/sex/dose Anon. 1985a	Diflufenican Batch 20 Purity 98.1% Oral, diet 0, 20, 100, or 500 ppm (equivalent to 0, 1.6/1.7, 8.0/8.7 and 38.1/44.3 mg/kg bw/ day in males and females) 13 weeks	<u>Cat 1 = 10</u> <u>Cat 2 = 100</u>	<u>Clinical signs / deaths / ophthalmology</u> Two deaths during blood sampling: one control female and one top dose male (not treatment related). Alopecia in 5 females at top dose. <u>1.6 / 1.7 mg/kg bw/day</u> No treatment-related effects observed. <u>8.0 / 8.7 mg/kg bw/day</u> ↓ ** bodyweight females (-19.5%) <u>38.1 / 44.3 mg/kg bw/day</u> Alopecia in 5 females ↓ ** bodyweight females (-19.5%) ↓* bodyweight males (-9.5%) NOAEL = males 1.6 mg/kg bw/day; females 1.7 mg/kg bw/day LOAEL = males 8 mg/kg bw/day; females 8.7 mg/kg bw/day based on decreased bodyweight, body weight gain & food consumption. Note: Ovaries, testes and the pituitary gland were analysed for weight change and histopathology.
Oral OECD 408 (1981) GLP Fischer 344 rats 35/sex/dose Anon. 1987a Anon. 1987b	Diflufenican Batch No. B18 Purity 98.4%. Oral, diet 0, 5, 25, 250 or 2500 ppm (equivalent to 0, 0.36/0.40, 1.80/2.01, 18.46/20.48 and 185.2/207.9 mg/kg bw/day in males and females) 13 weeks + 4 and 8 weeks recovery period	<u>Cat 1 = 10</u> <u>Cat 2 = 100</u>	<u>0.36 / 0.40 mg/kg bw/day (0.38 mg/kg bw/day combined)</u> No treatment-related effects observed. <u>1.8 / 2.01 mg/kg bw/day (1.91 mg/kg bw/day combined)</u> No treatment-related effects observed. <u>18.46 / 20.46 mg/kg bw/day (19.47 mg/kg bw/day combined)</u> ↓ *** bodyweight gain both sexes (7.8 – 10% reduction compared to controls for males and females respectively); reversible during recovery phase. <u>185.2 / 207.9 mg/kg bw/day (196.6 mg/kg bw/day combined)</u> ↓*** bodyweight gain both sexes (17.4 and 24% reduction compared to controls for males and females respectively); reversible during recovery phase. ↑** relative liver weight in both sexes (>10%). ↑ in hepatic cellularity (hypertrophy) in males (minimal or slight severity in all cases). Reversible. NOAEL = males 18.46 mg/kg bw/day; females 20.48 mg/kg bw/day; combined = 19.47 mg/kg bw/day. LOAEL = males 185.2 mg/kg bw/day; females 207.9 mg/kg bw/day;

			combined = 196.6 mg/kg bw/day based on decreased bodyweight, bodyweight gain & food consumption, related clinical chemistry changes and increased liver weights & liver hypertrophy. Note: Ovaries, uterus (with cervix), testes and the pituitary gland were analysed for weight change and histopathology.
Oral OECD 408 (Sept 1998) GLP Wistar HsdCpd: WU rats 10/sex/dose Anon. 2002a	Diflufenican Batch/Receipt No.: 7 Purity 98.8 % Main study 0, 150, 750, 3750 ppm (equivalent to 0, 11.4/13.1, 56.7/63.8, 280.4/312.0 mg/kg bw/day in males and females) Recovery 0 and 3750 ppm (equivalent to 0/0 and 286.7/304.5 mg/kg bw/day in males and females) 90 days treatment Control and top dose groups were maintained on untreated diet for a further 28 days (recovery period)	<u>Cat 1 = 10</u> <u>Cat 2 = 100</u>	<u>Clinical findings</u> No deaths. Local alopecia in seven females (1 control, 1 control-recovery, 2 at 3750 ppm, and 3 at 3750 ppm recovery group). One male in high dose group had a subcutaneous mass (fibrosarcoma). <u>11.4 / 13.1 mg/kg bw/day</u> No treatment-related effects observed. <u>56.7 / 63.8 mg/kg bw/day</u> ↓**food consumption, bodyweight and bodyweight gain both sexes <u>280.4 / 312.0 mg/kg bw/day (90 days treatment) and 286.7/304.5 mg/kg bw/ day (recovery)</u> ↓** bodyweight and bodyweight gain both sexes; ↑** bodyweight gain both sexes recovery week 13-17. Food consumption in females remained significantly below control levels during the recovery period. NOAEL = males 11.4 mg/kg bw/day; females 13.1 mg/kg bw/day LOAEL = males 56.7 mg/kg bw/day; females 63.8 mg/kg bw/day based on decreased bodyweight, body weight gain & food consumption. Note: Ovaries, uterus, testes and epididymides were analysed for weight change and histopathology.
Oral JMAF (1985) Compliant with OECD 408 (1981) GLP B6C3F1 mice 10/sex/dose Anon. 1993	Diflufenican Batch DA 826 Purity 97.9% Oral, diet 0, 500, 5000 or 20 000 ppm (equivlaent to 0, 79.0/104.2, 825.5/1024.0 and 3598.0/4002.0 mg/kg bw/day	<u>Cat 1 = 10</u> <u>Cat 2 = 100</u>	<u>Clinical signs / deaths - ophthalmology</u> No deaths occurred during the study and there were no obvious signs of toxicity or ophthalmologic anomalies. <u>79 / 104.2 mg/kg bw/day</u> No treatment-related effects observed. <u>825.5 / 1024.0 mg/kg bw/day</u> ↓ bodyweight gain males* and females** ↑ platelets**, AP**, cholesterol*** in males; ↑ AP***; ↓ glucose** in females ↑** relative liver weight in both sexes (> 30%); hepatocytic hypertrophy (7/10 compared to 0/10 in controls) in males only. <u>3598 / 4002 mg/kg bw/day (largely exceeding OECD recommended</u>

	in males and females) 13 weeks		limit dose) ↓ bodyweight gain males** and females** ↑ platelets***, AP***, ALT**, Protein*; ↓ glucose**, cholesterol** in males; ↑ leucocytes**, AP***; ↓ glucose**, cholesterol* in females ↑** relative liver weight both sexes (> 50%); hepatocytic hypertrophy (10/10 compared to 0/10 in controls) in males only. NOAEL = males 79 mg/kg bw/day; females 104.2 mg/kg bw/day LOAEL = males 825.5 mg/kg bw/day; females 1024 mg/kg bw/day based on decreased body weight gain & increased liver weight. Note: Ovaries, uterus (with cervix), testes and the pituitary gland were analysed for weight change and histopathology.
Oral OECD 409 (1981) GLP Beagle dogs 4/sex/dose Anon. 1984c	Diflufenican Batch B12 Purity 97.4%. Oral, gavage 0, 250, 500, and 1000 mg/kg bw/day 13 weeks	Cat 1 = 10 Cat 2 = 100	Clinical signs / deaths / Ophthalmology No deaths reported. Treatment-related incidence of vomiting: total dog-days of emesis were 0, 2, 14 and 47 for treatment groups 0, 250, 500 and 1000 mg/kg bw, respectively. 250 mg/kg bw/day No treatment-related effects observed. 500 mg/kg bw/day ↓** bw gain in females 1000 mg/kg bw/day ↓** bodyweight gain in females ↓* relative thymus and lung weight in females only NOAEL < 250 mg/kg bw/day LOAEL = 250 mg/kg bw/day based on treatment-related incidence of vomiting. Note: Ovaries, testes and the pituitary gland were analysed for weight change and histopathology.
Oral Compliant with OECD 409 (1981) GLP Beagle dogs 5/sex/dose Anon. 1987c	Diflufenican Batch FDS 4473 Purity 97.4% Oral, capsulated, in diet 0, 100, 300 or 1000 mg/kg bw/day 52 weeks	Cat 1 = 2.5 Cat 2 = 25	Clinical signs / deaths / Ophthalmology No notable effects observed. 100 mg/kg bw/day No treatment-related effects observed. 300 mg/kg bw/day ↑** relative liver weight and ↑*** cholesterol in females 1000 mg/kg bw/day ↑*** relative liver weight and ↓** AP in females ↑* relative liver weight in males with occasional statistical change in cholesterol NOAEL = 100 mg/kg bw/day LOAEL = 300 mg/kg bw/day based on increased liver weights, cholesterol & alkaline phosphatase.

			Note: Ovaries, uterus (with cervix), testes and the pituitary gland were analysed for weight change and histopathology.
Oral Broadly compliant with OECD 453 (1981) GLP F-344 rats 50/sex/dose Satellite groups: 30/sex/dose Anon. 1987d	Diflufenican Batch B18 Purity 98.4% Oral, diet 500, 2500, and 12500 ppm (equivalent to 23.27/27.78, 119.6/142.5 and 614.0/749.0 mg/kg bw/day) 104 weeks	Cat 1 = 1.25 Cat 2 = 12.5	Clinical signs / deaths / survival No notable effects observed. 23.27 / 27.78 mg/kg bw/day ↓** bodyweight gain in females (5% reduction compared to controls). 119.6 / 142.5 mg/kg bw/day ↓*** bodyweight gain both sexes (13 / 20% reduction in males / females respectively and compared to controls). 614 / 749 mg/kg bw/day ↓*** bodyweight gain both sexes (18 / 33% reduction in males / females respectively and compared to controls). ↑*** relative liver weight and ↓** AP in females ↑* relative liver weight males with occasional statistical change in cholesterol NOAEL = 100 mg/kg bw/day LOAEL = 300 mg/kg bw/day based on increased liver weights, cholesterol & alkaline phosphatase (AP). Note: Ovaries, uterus (cornua and cervix), testes and the pituitary gland were analysed for weight change and histopathology.
Oral Broadly compliant with OECD 453 (1981) GLP B6C3F1 hybrid mice 52/sex/dose Satellite groups: 40/sex/dose Anon. 1986	Diflufenican Batch B18 Purity 98.4% Oral, diet 0, 500, 2500, or 12500 ppm (equivalent to 0, 62.2/73.3, 321.7/384.4 or 1618.0/1989.0 mg/kg bw/day in males and females). 105 weeks	Cat 1 = 1.25 Cat 2 = 12.5	Clinical signs / deaths / survival Survival rates independent to treatment. Satellite study: Majority of deaths attributed to blood sampling method (week 25). 62.2 / 73.6 mg/kg bw/day ↓* bodyweight gain both sexes (5.3 - 8.7% reduction in males / females respectively and compared to controls). 321.7 / 384.4 mg/kg bw/day ↓*** bodyweight gain both sexes (11.9 - 17.4% reduction in males / females respectively and compared to controls). ↓ Cholesterol males* (53% reduction compared to controls) females** (57% reduction compared to controls) ↑ relative liver weight in males* (20% increase compared to controls) and females** (35% increase compared to controls) at week 104 ↑** hepatic hypertrophy in males** (7/10 slight to moderate) at week 52 compared to 0/10 in controls Seminal vesicle: ↑* incidence in reduction of secretion (19% increase compared to controls) ↓* distended with secretion (23% reduction compared to controls) 1618 / 1989 mg/kg bw/day ↓*** bodyweight gain both sexes (18.5/ 28.2% reduction in males / females respectively and compared to controls). ↓ Cholesterol males* (48% reduction compared to controls) females** (49% reduction compared to controls) ↑ relative liver weight in males (11% increase compared to controls) and females** (68% increase compared to controls) ↑*** hepatic hypertrophy in males (9/10 moderate to marked) week 52 compared to 0/10 controls ↑ relative pituitary weight females** (29% increase compared to

			controls) at week 104 Seminal vesicle: ↑* incidence in reduction of secretion (39% increase compared to controls) ↓* distended with secretion (25% reduction compared to controls) NOAEL = 62.2 / 73.6 mg/kg bw/day LOAEL = 321.7 / 384.4 mg/kg bw/day based on reduced bodyweight gain, clinical chemistry changes, increased liver weight (females), reduced seminal vesicle secretion. Note: Ovaries, uterus (with cervix), testes and the pituitary gland were analysed for weight change and histopathology.

AP = alkaline phosphatase
ALT = alanine aminotransferase
Statistically significant at: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

10.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The short-term toxicity of diflufenican was addressed in a number of studies conducted in the rat, mouse and dog. Moreover two combined oncogenicity and toxicity studies in rats and mice were also conducted for a period of 104 weeks. In most of the studies the ovaries, testes and pituitary gland were collected and weighed. More occasionally the uterus and cervix were also analysed for weight changes. The studies presented in this section provide supporting/additional information on the potential of diflufenican to cause adverse effect on the reproductive function (Please refer to Section 10.8.1 Adverse effects on sexual function and fertility). For each study the treatment-related effects relevant to their respective guidance cut-off values for STOT-RE classification are described below.

In the rat

In a 1983 GLP study, Sprague Dawley rats (5/sex/dose) were administered doses of 0, 400, 800, and 1600 mg/kg bw of diflufenican via oral gavage daily for 2 weeks. Food consumption and bodyweight development were normal throughout the course of the study. A dose-dependent increase in bilirubin in males was noted reaching statistical significance at a dose that exceeded the guidance value for STOT-RE classification for this study. Reproductive organ weight data and histopathological findings did not suggest any treatment-related effect on the reproductive system up to 1600 mg/kg bw/day.

In a 2001 GLP range-finding study Wistar rats (6/sex/dose) were administered diflufenican in the diet at doses of 0, 27.5-27.2, 128.9-134 and 674.2-668.7 mg/kg bw/day for 28 days. At 134 mg/kg bw/day (below the guidance cut-off value for STOT-RE category 2 classification) food consumption and body weight gain were significantly lower when compared to the control group throughout the treatment period in females only. At 27.2 mg/kg bw/day (below the guidance value for STOT-RE category 1 classification) food consumption only was significantly reduced in females. The decreases in body weight gains were possibly due to the unpalatable nature of the test item as indicated by a decrease in food consumption. No other relevant findings were noted such as for the reproductive organs analysed in this study at any dose level.

In a 1984 study that was non-GLP but generally compliant with OECD guideline 408, Sprague Dawley rats (15/sex/dose) were administered diets containing diflufenican at concentrations of 0, 32.8-37.5, 335-383 or 3448-3726 mg/kg bw/day for 13 weeks. The top dose clearly exceeded to limit dose recommended in the test guideline. Statistically significant reductions in bodyweights were seen from 37.5 mg/kg bw/day in males (below the guidance value for STOT-RE category 2 classification). However there was histopathological evidence of chronic respiratory disease in the majority of control and top dose animals which questions the validity of the study to provide relevant toxicological information on repeated exposure to diflufenican.

In a 1985 study that was non-GLP but compliant with OECD guideline 408, Sprague Dawley rats (15/sex/dose) were fed diets that contained 0, 1.6-1.7, 8.0-8.7, or 38.1-44.3 mg/kg bw/day diflufenican (all below the guidance value for classification in this study) for 13 weeks. Alopecia was evident in 5/15 females at 44.3 mg/kg bw/day. Bodyweight gain was statistically significantly reduced in males in the highest dose group towards the latter part of the study. In females, food consumption and bodyweight gain were reduced at 8.7 mg/kg bw/day and above for most of the duration of the study. Overall decreased bodyweight, body weight gain and food consumption were observed at doses below the guidance cut-off value for STOT-RE category 2 classification. No adverse effects were noted for the reproductive organs analysed in both sexes at all doses in this study.

In a 1987 GLP study F344 rats (35/sex/dose) were administered diet containing 0, 0.38, 1.91, 19.47 or 196.6 mg/kg bw/day diflufenican for 13 weeks. Recovery groups were maintained for a further 4 or 8 weeks without diflufenican. At doses below the guidance value for STOT-RE category 2 classification (19.47 mg/kg bw/day) bodyweight gains were statistically significantly reduced from controls in both sexes; however this effect was reversible. At 196.6 mg/kg bw/day (above the guidance value for STOT-RE category 2 classification) a statistical change in relative liver weight was observed in both sexes accompanied with a slightly increased incidence of hepatic hypertrophy in males; however all this appeared to be reversible. No other relevant findings were noted such as for the reproductive organs at any dose level.

In a 2001 GLP study, Wistar rats (10/sex/dose) were administered diflufenican in diet at doses of 0, 11.4-13.1 , 56.7-63.8 and 280.4-312 mg/kg bw/day for 90 days. Recovery groups were maintained on untreated diet for a further 28 days. The only effects observed at doses below the guidance value for STOT-RE classification were a statistically significantly reduction in food consumption, bodyweight and body weight gain at 56.7 mg/kg bw/day and above for both sexes. Recovery groups showed significant increases in bodyweight gain compared to controls, even though food consumption in females at 312 mg/kg bw/day remained significantly below control levels during the recovery period. No other relevant findings were observed such as for the reproductive organs analysed (ovaries, uterus, testes and epididymides) at any dose level.

In a 1987 GLP study, F-344 rats (50/sex/dose) were administered diflufenican in diet at doses of 0, 23.27-27.78, 119.6-142.5 and 614-749 mg/kg bw/day (all above the guidance value for classification in this study) for 104 weeks(. The only effect observed at doses close to the guidance value for STOT-RE classification (23.27-27.78 mg/kg bw/day) was a statistically significantly reduction in body weight gain in females only; however the reduction was slight compared to control groups(≈5%). Liver changes were observed at top doses. No other relevant findings were observed such as for the reproductive organs analysed (ovaries, uterus, testes and epididymides) at any dose level.

In the mouse

In a 1993 GLP study, B6C3F1 hybrid mice (10/sex/dose) were administered diet containing 0, 79.0-104.2, 825.5-1024 or 3598-4002 mg/kg bw/day diflufenican for 13 weeks (Eddie, M., 1993). It is to be noted that the top dose greatly exceeded the limit dose of the test guideline. All adverse effects observed in this study (decreased body weight and increased liver weight) were seen at doses largely exceeding the guidance value for STOT-RE classification. No notable effects were observed in the reproductive organs investigated in this study at all dose levels.

In a 1986 GLP study, B6C3F1 hybrid mice (52/sex/dose) were administered diflufenican in diet at doses of 0, 321.7-384.4 and 1618-1989 mg/kg bw/day (all above the guidance value for classification in this study) for 104 weeks (). The only effect observed at the lowest dose (62.2-73.6 mg/kg bw/day) was a statistically significantly reduction in body weight gain in females only; however the reduction was slight compared to control groups (5.3 and 8.7% for males and females respectively). Liver changes were observed at top doses. No other relevant findings were observed such as for the reproductive organs analysed (ovaries, uterus, testes and epididymides) at any dose level.

In the dog

In a1984 GLP study beagle dogs (4/sex/dose) were administered diflufenican daily at doses of 0, 250, 500, and 1000 mg/kg bw/day by oral gavage for 13 weeks, all of which exceeded the guidance value for STOT RE for a 90-day rat study. No treatment related effects were observed at 250 mg/kg bw/day. The principal clinical observation was a treatment-related increased incidence of vomiting and significant decreases in bodyweight gain in females at 500 and 1000 mg/kg bw. No reproductive organs such as the ovaries and testes were affected by the administration of diflufenican at all dose levels.

In a 1987 GLP study beagle dogs (5/sex/dose) were administered diflufenican via the oral route (capsules) on a daily basis at doses of 0, 100, 300 or 1000 mg/kg bw/day (all exceeding the guidance value for STOT RE classification) for 52 weeks (West, H. A., 1987c.). No treatment-related effects were observed at 100 mg/kg bw/day. Liver effects were seen from 300 mg/kg bw/day. No reproductive organs such as the ovaries, uterus and testes were affected by the administration of diflufenican at all dose levels.

No data for short-term dermal and inhalation toxicity were available.

10.10.2 Comparison with the CLP criteria

Not considered in this report. Information on repeated dose toxicity has been provided as support to the section on reproductive toxicity only.

10.10.3 Conclusion on classification and labelling for STOT RE

Not considered in this report.

10.11 Aspiration hazard

This endpoint has not been considered in this report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 13: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Hydrolysis			
Not reported	22°C. 30 day study at pH 5, 7 and 9. Diflufenican reported to be stable to hydrolysis at all pH tested based on levels not declining by more than 10% over the study duration.	Study predates GLP requirements.	Reeves, G. L.; Savege, E. A. (1985)
Not reported	50°C and 70°C. 30 day study at pH 5, 7 and 9. Diflufenican reported to be stable to hydrolysis at all temperatures and pH tested based on levels not declining by more than 10% over the study duration.	Study methodology similar to Reeves, G. L.; Savege, E. A. (1985) but at higher temperatures. Study predates GLP requirements.	Reeves, G. L.; Savege, E. A. (1986)
Procedures for assessing the environmental fate	50°C. 5 day incubation at pH 4, 7 and 9. Diflufenican reported		Juozenaite, A. (2008)

Method	Results	Remarks	Reference
and ecotoxicity of pesticides; Society of Environmental Toxicology and Chemistry (SETAC- Europe, 1995) GLP compliant	to be stable to hydrolysis at all temperatures and pH tested.		
Photolysis (Aqueous, Direct)			
J-MAFF 2-6-2 (2001) EC Method C7 (1994). GLP compliant	25°C, xenon lamp, wavelengths <290 nm filtered out, continuous illumination for 17 days, equivalent to 33.15 days natural sunlight at 50°N in the UK.. Single first order DT50 calculated as 133 d under artificial illumination (less than 10% degradation in 17 days. No significant degradation in dark control. Quantum yield was calculated as 2.75 x 10^{-5}. Based on the results of Simmonds and Mills (2002a), using the method of Frank and Klöpffer (1988), half lives in the top few centimetres of a natural aquatic system using solar irradiance data appropriate to central European latitudes (52°N) were calculated. Based on these assumptions the half-life for diflufenican ranged from 2500 h (104 d) in June to 46000 h (1875 d) in December.	When compared to triggers in OECD 316 guidance, direct photolytic degradation is not considered to be a significant process for diflufenican	Simmonds M.B. & Mills E.A.M. (2002a) and Simmonds M.B. & Mills E.A.M. (2003a) Buntain I.G. (2003a)
Society of Environmental Toxicology and Chemistry (SETAC). Procedures for assessing the environmental fate and ecotoxicity of pesticides. Ed. M.R. Lynch. Section 10: Aqueous photolysis (1995). OECD Guideline for Testing of Chemicals. Proposal for new guideline. Phototransformation of Chemicals in	25°C, xenon lamp, wavelengths <290 nm filtered out, continuous illumination for 13 days, equivalent to 30.2 days natural summer sunlight at 40°N. At study end, 63.3% of applied radioactivity remained as diflufenican. In dark control 95.2% remained as diflufenican at study end. SFO DT50 under continuous illumination was 18.85 days, equivalent to 44.06 days of summer sunlight at 40°N.	When compared to triggers in OECD 316 guidance, direct photolytic degradation is not considered to be a significant process for diflufenican.	Brands (2008)

Method	Results	Remarks	Reference
Water – Direct Photolysis. Draft Document (December 3, 2007). GLP compliant			
OECD 316, 2008 Phototransformation of Chemicals in Water – Direct Photolysis. GLP compliant	25°C, xenon lamp, wavelengths <290 nm filtered out, continuous illumination for 10 days, equivalent to 30 days natural sunlight at 55°N. Three concentrations of diflufenican tested, 10, 17,5, 25 µg/l. At study end, 26.4 – 34.5% of applied radioactivity remained as diflufenican. In dark control virtually all radioactivity remained as diflufenican at study end. SFO DT50 under continuous illumination was 5.31 – 6.89 days; based on quantum yield (mean value 3.52 x 10^{-5}) the environmental half-life was estimated to be equivalent to be 64.3 days (in June) – 47.2 years (in December) at 55°N.	Some variability in recovery of radioactivity. When compared to triggers in OECD 316 guidance, direct photolytic degradation is not considered to be a significant process for diflufenican.	Derz, K. (2012)
Society of Environmental Toxicology and Chemistry (SETAC). Procedures for assessing the environmental fate and ecotoxicity of pesticides. Ed. M.R. Lynch. Section 10: Aqueous photolysis (1995). GLP compliant	25°C, xenon lamp, wavelengths <290 nm filtered out, continuous illumination for 7 days, equivalent to 25.8 days natural summer sunlight at 40°N. At study end, 82.6% of applied radioactivity remained as diflufenican. In dark control there was no decline in diflufenican at study end. A half-life of 42 days was calculated under continuous illumination, equivalent to a half-life of 156 days natural summer sunlight at 40°N .	When compared to triggers in OECD 316 guidance, direct photolytic degradation is not considered to be a significant process for diflufenican.	Unsworth, R. (2007)
Photolysis (Aqueous, Indirect)			
Japanese Guidelines concerning the Photolysis in Water J-MAFF 2-6-2, June 26, 2001 and using the Guidance Notification 13	Photolysis in sterilised natural water. 25°C, xenon lamp, wavelengths <290 nm filtered out, continuous illumination for 17 days, equivalent to 81 days natural spring sunlight in Japan.		Mills, E. A. M. & Simmonds, M.B. (2002)

Method	Results	Remarks	Reference
Seisan No 3986, October 10, 2001. GLP compliant	At study end, 87.7% of applied radioactivity remained as diflufenican. In dark control there was no decline in diflufenican at study end. The half-life was 80 days under continuous illumination, equivalent to a half-life of 388 days in Japan under spring conditions at 35°N.		
Ready biodegradability			
OECD No. 301D, Closed Bottle Test GLP compliant	Diflufenican 5.2% biodegradation after 28 days. Sodium benzoate reference 90.1% biodegradation based on biochemical oxygen demand.	Diflufenican classed as not readily biodegradable.	Lebertz, H., (1989)
OECD No. 301B: Ready Biodegradability GLP compliant	Diflufenican 9 - 21% biodegradation after 29 days. Sodium acetate reference 85% biodegradation based on $ThCO_2$.	Diflufenican classed as not readily biodegradable.	Desmares-Koopmans, M. J. E., (2008)
Biodegradation (in simulated water or water/sediment systems)			
OECD 309 GLP compliant	Pelagic fresh water system, 20°C, dark, 63 day duration. Diflufenican half-life >1000 d in both high and low dose treatments at 20°C. Correction of degradation rates to more environmentally realistic temperatures, e.g. 12°C, has not been undertaken since this would only increase the existing DT50s and so would not change the overall 'rapid degradability' determination.	Reference substance (benzoic acid degraded to <LOD after 3 days (10.7% as CO_2 at 3 days).	Hein & Kasel (2016)
OECD 309 GLP compliant	Pelagic fresh water system, 20°C, dark, 90 day duration. No decline in diflufenican concentration was observed over the 90 day study duration; consequently half-lives could not be calculated.	Reference substance (benzoic acid) produced 69.9% CO_2 in 13 days. Normally the reference substance would be expected to completely degrade within 2 weeks. However, it is accepted that the microbial population was viable.	Ilieva (2016b)
BBA IV: 5-1 (1990) GLP compliant	Two natural water/sediment systems, 20°C, dark, 121 days duration. Up to 71 – 74% of applied radioactivity as diflufenican partitioned to sediment at 8 – 44 days after treatment. 38.8 – 62.3% of applied radioactivity as diflufenican remaining in total system at study end,	Study similar to OECD 308.	Knoch, E. (1996)

Method	Results	Remarks	Reference
	mainly in sediment. Decline in whole system was best described by bi phasic kinetics; DT50 86 - >1000 days; DT90 341 - >1000 days.		
BBA IV: 5-1 (1990) SETAC Procedures for Assessing he Environmental Fate and Ecotoxicity of Pesticides, 1995, Part 1, Section 8.2 GLP compliant	Two natural water/sediment systems, 20°C, dark, 365 days duration. Up to 67 - 74% of applied radioactivity as diflufenican partitioned to sediment at 14 days after treatment. 34.9 – 56.2% of applied radioactivity as diflufenican remaining in total system at study end, mainly in sediment. At 120 days, 53.5 – 67.1% of applied radioactivity remained as diflufenican in the total system. Decline in whole system was best described by bi phasic kinetics; DT50 180 - >1000 days; DT90 >1000 days.	Study similar to OECD 308.	Crowe, A. (2003)
SETAC Procedures for Assessing he Environmental Fate and Ecotoxicity of Pesticides, 1995, Part 1, Section 8.2 GLP compliant	Two natural water/sediment systems, 20°C, dark, 100 days duration. Up to 67 - 82% of applied radioactivity as diflufenican partitioned to sediment at 14 – 59 days after treatment. 39.8 – 80.0% of applied radioactivity as diflufenican remaining in total system at study end, mainly in sediment. Decline in whole system was best described by bi phasic kinetics; DT50 86 - >1000 days; DT90 422 - >1000 days.	Study similar to OECD 308.	Unsworth, R. (2006)
OECD Guideline No. 308 SETAC Procedures for Assessing he Environmental Fate and Ecotoxicity of Pesticides, 1995, Part 1, Section 8.2 EPA Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, 162-4, 1985 GLP compliant	Two natural water/sediment systems, 20°C, dark, 179 days duration. Up to 78 - 86% of applied radioactivity as diflufenican partitioned to sediment at 27 – 103 days after treatment. 69.3 – 80.3% of applied radioactivity as diflufenican remaining in total system at study end, mainly in sediment. At 103 days, 74.1 – 84.2% of applied radioactivity remained as diflufenican in the total system. Decline in whole system was		Mamouni, A. (2003)

Method	Results	Remarks	Reference
	best described by bi phasic kinetics; DT50 998 - >1000 days; DT90 >1000 days.		
OECD Guideline No. 308 GLP compliant	Two natural water/sediment systems, 20°C, dark, 102 days duration. Up to 77 - 86% of applied radioactivity as diflufenican partitioned to sediment at 14 – 56 days after treatment. 79.2 – 83.9% of applied radioactivity as diflufenican remaining in total system at study end, mainly in sediment. Decline in whole system was best described by bi phasic kinetics; DT50 >1000 days; DT90 >1000 days.		Adam, D. (2008)

11.1.1 Ready biodegradability

Two ready biodegradability studies conducted on diflufenican were submitted, one to OECD 301D and one to OECD 301B guidelines (Lebertz, H., (1989) and Desmares-Koopmans, M. J. E., (2008)). Both studies were conducted appropriately and to GLP and they are considered to be reliable. In both studies, diflufenican showed very little degradation, 5.2 – 21% biodegrading after 28-29 days. In contrast, the reference substances showed high levels of biodegradation (90.1% based on BOD in one study, 85% based on $ThCO_2$ in the other) indicating the systems were microbially viable. Both studies conclude that diflufenican is not classified as readily biodegradable.

11.1.2 BOD_5/COD

No BOD_5/ COD were reported for diflufenican.

11.1.3 Hydrolysis

Three aqueous hydrolysis studies were submitted (Reeves, G. L.; Savege, E. A. (1985); Reeves, G. L.; Savege, E. A. (1986); Juozenaite, A. (2008)). The older two studies were conducted prior to GLP requirements and to no recognised study guideline. However, they appear to be well conducted and give reliable information on hydrolysis at 22°C, 50°C and 70°C and at each of three pH, pH 5, 7 and 9. In these two studies conducted over 30 days, there was less than 10% degradation of diflufenican observed under each combination of temperature and pH, the studies concluding that diflufenican is stable to hydrolysis The third (Juozenaite, A., 2008) is a modern GLP compliant study conducted to SETAC guidelines (similar to OECD 111) and is considered to be reliable. The study was conducted for 5 days at 50°C and each of pH 4, 7 and 9. Diflufenican was reported to be stable to hydrolysis at each pH tested at 50°C. All three studies showed consistent behaviour for diflufenican.

11.1.4 Other convincing scientific evidence

No information.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No information.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No information.

11.1.4.3 Water and water-sediment degradation data (including simulation studies)

Aerobic mineralisation in surface water

Two reliable aerobic mineralisation in surface water studies, conducted to OECD 309 and GLP compliant, are available for diflufenican (Hein & Kasel (2016); Ilieva (2016b)). Both studies used pelagic fresh water systems at 20°C in the dark; two dose rates were used in each study. Both studies showed degradation of benzoic acid reference standards. However, there was virtually no degradation of diflufenican, a half-life for primary degradation of >1000 days being calculated in the study of Hein & Kasel (2016). In the study conducted by Ilieva (2016b), no decline in diflufenican concentration in the water-phase was observed and no half-life could be calculated. There was a maximum of 0.5% of applied radioactivity recovered as CO_2 at study end. In the study by Ilieva (2016b)), where very limited degradation of diflufenican was seen, up to 4 unidentified metabolites were observed, the highest of these occurring at a maximum of 4.2% of applied radioactivity. Both studies showed consistent results for diflufenican.

Water/sediment studies

Five valid water/sediment studies are available for diflufenican (Knoch, E. (1996); Crowe, A. (2003); Unsworth, R. (2006); Mamouni, A. (2003); Adam, D. (2008)). All were conducted to OECD 308 or other internationally recognised guidelines very similar to OECD 308. The studies were all conducted to GLP and are considered to be reliable. The studies were of variable duration, ranging from 100 – 365 days, but all were conducted in the dark at 20°C. Each study was conducted on two contrasting fresh water/sediment systems. All systems showed significant partitioning of diflufenican to sediment, 67 – 86% of radioactivity being recovered as diflufenican in sediment at 14 – 103 days after treatment. Decline of diflufenican in the water layer was primarily due to partitioning to sediment. Diflufenican underwent slow primary degradation in the whole system with significant quantities of unchanged diflufenican remaining at the end of the study. For comparison purposes, the amount of diflufenican remaining in the total system (i.e. water + sediment) at 100 – 121 days ranged from 38.8 – 85.3% of applied radioactivity. The kinetics of primary degradation in the whole system (i.e. based on decline of extracted diflufenican residues) were typically biphasic with DT50 values ranging from 86 - >1000 days and DT90 values of 341 - >1000 days at 20°C. Correction of degradation rates to more environmentally realistic temperatures, e.g. 12°C, has not been undertaken since this would only increase the existing DT50s and so would not change the overall 'rapid degradability' determination. CO_2 accounted for <0.05 – 4.5% at 100 – 121 days after treatment; unextracted radioactivity accounted for 2.8 – 22.3% of applied radioactivity at 100 – 121 days after treatment. All five studies showed relatively consistent behaviour for diflufenican. Two metabolites were formed in quantities greater than 5%. Metabolite AE B107137 (2-[3-(trifluoromethyl)phenoxy]pyridine-3-carboxylic acid) was formed at maxima of 7.7 – 46.0% of applied radioactivity in total system at 27 – 121 days after treatment. Metabolite AE C522392 (2,4-difluoroaniline) was formed at maxima of 1.0 – 8.0% of applied radioactivity at 14 – 59 days after treatment. Other minor metabolites were observed at maxima of <3% of applied radioactivity in total system on an individual basis, with between one and four of these minor metabolites observed per water/sediment system.

11.1.4.4 Photochemical degradation

A number of different studies were conducted to explore the direct aqueous photolysis of diflufenican and to calculate the photolytic half-lives (Simmonds M.B. & Mills E.A.M. (2002a); Simmonds M.B. & Mills E.A.M. (2003a); Buntain I.G. (2003a); Brands (2008); Derz, K. (2012); Unsworth, R. (2007)). All studies were GLP compliant but used a variety of internationally accepted guidelines, including OECD guidance. All studies are considered reliable. All studies used pure water, xenon lamps with

appropriate filtering to remove wavelengths <290 nm and with continuous illumination at 25°C. Light energy and intensity differed between studies and comparison of light energy input under experimental conditions to natural sunlight was not standardised. In addition, study duration varied from 7 – 17 days. As a consequence, variable amounts of diflufenican remained unchanged at the end of the study (for example 82.6% of applied radioactivity remained as diflufenican in the 7 day study (Unsworth, R. 2007), and 26-34% remained as diflufenican in the 10 day study(Derz, K. 2012). Calculated half-lives were 44 – 156 days at 40 – 55°N in the summer. Whilst enhanced degradation was observed (virtually no degradation was seen in dark controls), degradation due to photolysis was relatively slow.

A further study on indirect aqueous photolysis was also conducted (Mills, E.A.M. & Simmonds, M.B. (2002)). The study was conducted according to a Japanese guideline and was GLP compliant. The study used sterilised natural water but in an otherwise similar experimental procedure to the direct aqueous photolysis studies. The calculated environmental half-life for photolysis in this study was 388 days under Japanese spring conditions at 35°N.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

The available information on the behaviour of diflufenican in water (hydrolysis, photolysis, aerobic mineralisation in surface water and natural water/sediment studies) indicate that diflufenican is stable to hydrolysis, undergoes very slow primary degradation in non-sterile water conditions and has enhanced but slow degradation under illumination. Water/sediment studies indicate that significant partitioning from water into sediment occurs but primary degradation is slow in the entire water/sediment systems. Diflufenican is not readily biodegradable. Given that DT50s at the temperatures used in these studies (typically ≥ 20ºC) indicate that diflufenican was only slowly degraded, even slower degradation would be expected if these values were converted to 12ºC.

Overall, the information on degradation does not show that diflufenican is ultimately degraded, to > 70 % degradation within 28 days (equivalent to a half-life < 16 days), or transformed to non-classifiable degradants. Consequently, diflufenican is considered to be ‘not rapidly degradable’ according to CLP criteria.

11.3 Environmental fate and other relevant information

Soil adsorption

Adsorption/desorption of diflufenican was studied in 21 soils from four studies (Giraud and Plewa (1984); Simmonds and Brett (2006); Knight (2008a); Traub (2012a)). The studies were conducted to OECD 106 or similar guidelines and, and with the exception of the earliest study, were conducted to GLP. The earliest study pre-dated GLP requirements. The studies were considered to be reliable. Clay content ranged from 1.6 – 41.1 %, organic carbon content ranged from 0.64 – 3.6 % and pH from 4.1 – 7.7 % ($CaCl_2$) and 5.3 – 8.0 % (in water). The calculated adsorption coefficient K_{FOC} was in the range 531.6 - 7431 mL/g, geomean 2215 mL/g. The calculated arithmetic mean Freundlich exponent (1/n) was 0.87. There was a moderate negative relationship between K_{FOC} and pH. Overall, the results indicate diflufenican would be moderately to strongly adsorbed to soil or sediment. Water/sediment studies confirm this with high levels of partitioning into sediment.

Volatilisation

Diflufenican has a vapour pressure of 4.25 x 10^{-6} Pa at 25°C and Henry's Law constant calculated from vapour pressure and solubility of 1.8 x 10^{-2} $Pa.m^3.mol^{-1}$. This suggests that diflufenican is of low volatilisation potential and is unlikely to partition from water to the air.

11.4 Bioaccumulation

Studies have been performed to measure the bioaccumulation of diflufenican in fish, these studies are summarised in Table and further discussion is included in Section 11.4.2.

Table 14: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD 305; 28-day exposure phase, 14-day depuration phase, flow-through exposure	Whole fish: 1596 L Kg^{-1} (exposure at 3 µg a.s./L) 5 % lipid (whole fish): 1650 L Kg^{-1} (exposure at 3 µg a.s./L)	Study considered suitable for use in hazard classification.	Anon. 1998a
OECD 305; 21-day exposure phase, 14-day depuration phase, flow-through exposure	Whole fish: 1338 L Kg^{-1} (exposure at 3 µg a.s./L) 5 % lipid (whole fish): 2583 L Kg^{-1} (exposure at 3 µg a.s./L)	Study considered suitable for use in hazard classification.	Anon. 2008

11.4.1 Estimated bioaccumulation

Not applicable, measured partition coefficient and bioaccumulation test data available (see Section 11.4.2).

11.4.2 Measured partition coefficient and bioaccumulation test data

The measured Log K_{ow} for diflufenican is 4.2 at 20°C; this is greater than the CLP Log K_{ow} trigger of ≥ 4 and indicates a potential for bioaccumulation. Under the requirements of Reg. (EC) 1107/2009 (and the data requirements in Reg. (EU) 283/2013) measured estimates of bioconcentration in fish were provided in relation to diflufenican.

Two valid studies (Anon. 1998a; Anon. 2008) are available estimating the BCF for fish (see Table). Both studies are considered reliable and can be considered in relation to estimating the bioaccumulation potential. Brief summaries are included below:

Anon 1998a. (^{14}C)-Diflufenican: Bioaccumulation and metabolism in rainbow trout.

Bioaccumulation of diflufenican was investigated using Rainbow Trout (*Oncorhynchus mykiss*), according to the OECD 305 guideline and according to GLP. The test item was radio labelled [^{14}C]-diflufenican. The fish were exposed to a continual flow of dilution water for 28 days, at nominal treatment concentrations of 0.3 or 3.0 µg a.s./L (exposure phase). The fish were then exposed to a continuous flow of dilution water alone for a further 14 days (depuration period). A control was performed in parallel using the dilution water and the organic solvent used to prepare the treatment solutions. The study met the relevant validity criteria according to OECD 305.

Daily water samples were examined and demonstrated that the test item was maintained at between 0.28 - 0.39 µg a.s./L (mean 0.33 µg a.s./L) in the 0.3 µg a.s./L (nominal) treatment group and between 2.69 - 3.63 µg a.s./L (mean 3.03 µg a.s./L) in the 3.0 µg a.s./L (nominal) treatment group. On the first day of exposure, water concentrations had fallen significantly but thereafter returned to > 90 % of the nominal concentration. This decrease was interpreted as due to an accumulation of radioactivity into the fish, producing concentrations equivalent to 0.22 and 2.18 µg a.s./L at 0.3 and 3.0 µg a.s./L (nominal), respectively.

Low levels of radioactivity were detected in water from the test tanks during the first week of depuration. Otherwise levels of radioactivity in the depuration phase and in the control experiment were derived from data below the limit of reliable determination.

The principal radioactive component in water from the exposure phase was unchanged diflufenican. Measured concentrations of diflufenican in the experiment at 0.3 µg a.s./L, after 0, 7, 14, 21 and 28 days exposure were equivalent to 0.33, 0.28, 0.33, 0.26 and 0.29 µg a.s./L respectively. The corresponding values at the higher treatment level were equivalent to 3.08, 3.00, 3.09, 2.62 and 3.01 µg a.s./L. In addition, small quantities of the metabolite AE B107137 (1.4 %) were found at the higher exposure concentration after 28 days.

Tissue concentrations of total radioactivity increased to an apparent steady state after 8-11 days during the exposure phase. Tissue concentrations in fish sampled at the end of the exposure phase were measured in edible and inedible tissues. The nature of the radioactivity in the fish was measured and diflufenican constituted 86.2 to 100.6 % of the total radioactivity, the remaining radioactivity was either 'unextracted residues' or 'water acetonitrile-soluble radioactivity'.

The results of the accumulation and depuration phases, including the resulting bioconcentration factor (BCF) estimates, are summarised in Table 15.

Table 15: Accumulation and depuration of diflufenican and the resulting bioconcentration factor (BCF) estimates for *O. mykiss*

	0.3 µg a.s./L			3.0 µg a.s./L		
	Whole fish	Fillet	Viscera	Whole fish	Fillet	Viscera
Time to 90 % steady state (days)	7.8 – 8.0	7.8 – 8.0	7.8 – 8.0	9.7 – 11.0	9.7 – 11.0	9.7 – 11.0
Uptake rate constant (mg/kg fish)/(mg/L water)/day	379	230	552	356	266	463
Mean 28 d tissue concentrations (mg/kg)	0.429	0.269	0.626	4.439	3.118	6.347
Depuration rate constant ((mg/kg fish)/(mg/L water)/day)	0.298	0.289	0.290	0.223	0.238	0.209
Time to 50 % depuration (days)	2.3 – 2.4	2.3 – 2.4	2.3 – 2.4	2.9 – 3.3	2.9 – 3.3	2.9 – 3.3
14 day depuration level (µg/g)	0.010	0.006	0.015	0.123	0.074	0.190
Bio-concentration factor (BCF)	**1276**	798	1906	**1596**	1118	2214
5 % lipid normalised bioconcentration factor (BCF) *	**1300**	1100	1550	**1650**	1550	1800

* Calculated during the renewal of the active substance under Reg. (EC) 1107/2009 (circa 2018)
Concentrations of lipid were determined as 6.23 % (viscera), 3.57 % (fillet) and 4.90 % (whole fish)

The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Anon. 2008. Diflufenican: Bioconcentration in rainbow trout.

Bioaccumulation of diflufenican was investigated using Rainbow Trout (*O. mykiss*), according to the OECD 305 guideline and according to GLP. The test item was radio labelled [^{14}C]-diflufenican. The fish were exposed to a continual flow of dilution water for 21 days, at nominal treatment concentrations of 0.3 or 3.0 µg a.s./L (exposure phase). The fish were then exposed to a continuous flow of dilution water alone for a further 14 days (depuration period). An untreated control was performed in parallel. The study met the relevant validity criteria according to OECD 305.

Daily water samples were examined and demonstrated that the level of radioactivity in the tanks was maintained at a mean of 0.324 µg a.s./L in the 0.3 µg a.s./L (nominal) treatment group, and a mean of 3.08 µg a.s./L in the 3.0 µg a.s./L (nominal) treatment group. After extraction into ethyl acetate [^{14}C]-diflufenican

accounted for 95.4 - 99.4 % radioactivity in tank water at both dose levels, throughout the exposure period. The mean concentration of [^{14}C]-diflufenican in the tank water during the 21 day exposure period was 0.308 µg a.s./L at the low exposure level and 2.98 µg a.s./L at the high exposure level.

The concentration of total radioactivity in the tissues increased to an apparent steady state after 7 days during the exposure phase. Tissue concentrations in fish sampled at the end of the uptake phase were measured in edible and inedible tissues. No metabolites were identified that accounted for > 10% of the radioactivity in samples of fish tissue.

The results of the accumulation and depuration phases, including the resulting bioconcentration factor (BCF) estimates, are summarised in Table .

Table 16: Accumulation and depuration of diflufenican and the resulting bioconcentration factor (BCF) estimates for *O. mykiss*

	0.3 µg a.s./L			3.0 µg a.s./L		
	Whole fish	Edible	Non-edible	Whole fish	Edible	Non-edible
Time to 90 % steady state (days)	7					
Mean tissue concentrations (day 10 – 20) (µg/g)	0.372	0.143	0.492	4.11	1.92	5.25
Time to 50 % depuration (days)	1.5	1.0	1.5	1.5	1.0	1.5
14 day depuration level (µg/g)	0.005	0.001	0.006	0.027	0.010	0.037
Bio-concentration factor (BCF)	**1149**	440	1518	**1338**	626	1710
5 % lipid normalised bioconcentration factor (BCF)	**2218**	1833	2085	**2583**	2608	2349

Concentrations of lipid were determined as; 3.98 % (non-edible), 1.15 % (edible) and 2.68 % (whole fish) during the exposure phase; and 5.56 % (non-edible), 1.12 % (edible) and 3.48 % (whole fish) during the depuration phase.

The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Overall, the measured Log K_{ow} for diflufenican (at 4.2) is greater than the CLP Log K_{ow} trigger of ≥ 4 and the experimentally determined whole-fish, lipid-normalised BCF values exceed the CLP trigger of ≥ 500. The information available on diflufenican therefore indicates that it has a potential for bioaccumulation according to CLP criteria.

11.5 Acute (short-term) aquatic hazard

Studies available during the renewal of diflufenican as an active substance, circa 2018, under Reg. (EC) 1107/2009 are summarised in Table . All the listed studies have been conducted according to GLP. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes. Only studies submitted testing the technical substance, diflufenican, have been summarised below (formulation studies submitted in the context of the active substance renewal under Reg. (EC) 1107/2009 have not been considered further here).

(Note that the available acute toxicity data available for the degredants of diflufenican (AE B107137 and AE 0542291) considered relevant under Reg. (EC) 1107/2009 are summarised in Annex I. This information is included for information only, none of the degredants exhibit equivalent toxicity to diflufenican or would require hazard classification in isolation. And so are not considered to impact the hazard classification of diflufenican.)

Table 17: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Reference	Submitted for original approval (O) or renewal (N)
Fish					
OECD 203; 96-hours, static exposure	***Cyprinus carpio***	**diflufenican (purity 96.8 %)**	**LC_{50} > 98.5 µg /L (m.m.)[2]**	**Anon. 1998c**	**O**
OECD 203; 96-hours, static exposure	*Oncorhynchus mykiss*	diflufenican (purity 96.8 %)	LC_{50} > 108.8 µg /L (m.m.)[2]	Anon. 1998b	O
OECD 203; 96-hours, semi static exposure	*O. mykiss*	diflufenican (purity 98.8 %)	LC_{50} > 32.8 µg /L (g.m.)	Anon. 2007a	N
OECD 203; 96-hours, semi static exposure	*Pimephales promelas*	diflufenican (purity 98.8 %)	LC_{50} > 39.8 µg /L (g.m.)	Anon. 2007b	N
Aquatic invertebrates					
OECD 202; 48-hours, static exposure	***Daphnia magna***	**diflufenican (purity 96.8 %)**	**EC_{50} > 240 µg /L (m.m.)[2]**	**Odin-Feurtet, 1999f**	**O**
OECD 202; 48-hours, static exposure	*D. magna*	diflufenican (purity 98.8 %)	EC_{50} > 42 µg /L (g.m.)	Wilby, 2007e	N
Algae					
OECD 201; 72-hours, static exposure	***Raphidocelis subcapitata* (Formerly *P. subcapitata* and *S. capricornutum*)**	**diflufenican (purity 98.8 %)**	**E_rC_{50} = 0.6 µg /L (g.m.)**	**Wilby, 2007g**	**N**
OECD 201; 72-hours, static exposure	*Anabaena sp.*	diflufenican (purity 98.8 %)	E_rC_{50} > 43.7 µg /L (g..m.)	Wilby, 2007h	N
OECD 201; 72-hours, static exposure	*Navicula pelliculosa*	diflufenican (purity 96.7 %)	E_rC_{50} 4.3 µg /L (m.m.)	Hoberg, 1997a	O
OECD 201; 72-hours, static exposure	*N. pelliculosa*	diflufenican (purity 98.8 %)	E_rC_{50} = 6.08 µg /L (g.m.)	Wilby, 2007i	N
OECD 201; 72-hours, static exposure	*Mycrocystis aeruginosa*	diflufenican (purity 96.8 %)	E_rC_{50} > 98 µg /L (m.m.)	Hoberg, 1998f	O
OECD 201; 72-hours, static exposure	*Chlamydomonas rheinhardtii*	diflufenican (purity 100 ± 0.6 %)	E_rC_{50} = 44.9 µg /L (g.m.)	Juckeland, 2015a	N
OECD 201; 72-hours, static exposure	*Chorella vulgaris*	diflufenican (purity 100 ± 0.6 %)	E_rC_{50} = 139.5 µg /L (nom.)[3]	Juckeland, 2015b	N
OECD 201; 72-hours, static exposure	*Desmodesmus communis*	diflufenican (purity 100 ± 0.6 %)	E_rC_{50} = 21.5 µg /L (g.m.)[3]	Juckeland, 2015c)	N
Other aquatic plants					
US EPA FIFRA Guidelines 12-2	*Lemna gibba*	diflufenican (purity	E_rC_{50} > 40 µg /L (nom.)	Hoberg, 1998h	O

and 123-2; 14-days, static exposure		96.8 %)			
OECD 221; 7-days, semi static exposure	*Lemna minor*	diflufenican (purity 98.8 %)	E_rC_{50} = 40 µg /L (nom.)	Wilby, 2007k	N
OECD 239; 14-days, static exposure	*Myriophyllum spicatum*	diflufenican (purity 98.8 %)	E_rC_{50} > 4.04 µg /L (g.m.)	Hermes & Emmet, 2016	N
OECD 239; 14-days, static exposure	*M. spicatum*	diflufenican (purity 100 ± 0.6 %)	E_rC_{50} = 14.8 µg /L (g.m.)	Juckeland, 2015d	N
OECD 239; 14-days, static exposure	*M. spicatum*	diflufenican (purity 97.3 %)	E_rC_{50} > 101 µg /L (g.m.)	Kuhl, 2016a	N
Other aquatic organisms					
No guideline but adapted from OECD 203 and US EPA 850.1075; 48-hours, static exposure	*Xenopus laevis*	diflufenican (purity 99.2 %)	LC_{50} > 70.5 µg /L (m.m.)	Anon. 2013	N

Bold entries are the endpoints considered most suitable to set the hazard classification for the active substance for each group of organisms.
Note: Data owners have been identified in square brackets for all new studies; new in the context of the most recent renewal of diflufenican under Reg. (EC) 1107/2009, circa 2018.
[1] m.m. = measured concentration; g.m.= geometric mean measured concentration; nom. = nominal concentration; n.a. = not available
[2] Some treatment concentrations were above the limit of solubility, for at least part of the test duration.
[3] This value is extrapolated beyond the highest tested treatment concentration.

11.5.1 Acute (short-term) toxicity to fish

Acute studies were provided both for the original approval for diflufenican under Dir. 91/414/EEC, and for the renewal of the active under Reg. (EC) 1107/2009 (circa 2018). Those studies considered acceptable are summarised below.

Anon.1998c. Diflufenican: Acute toxicity (96-hour) to common carp (*Cyprinus carpio*) under static conditions.

The acute toxicity of diflufenican to *C. carpio* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal concentrations of 0 (control), 9.4, 18.8, 37.5, 75.0 and 150 µg a.s./L (a solvent control using DMF was also tested). The 150 µg a.s./L treatment group was above the limit of solubility (film on the surface of the test solution), all other treatments were visually soluble. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore mean measured treatment concentrations were established at 6.8, 14.4, 26.8, 52.2 and 98.5 µg a.s./L (based on measurements at the test start and termination), the mean measured treatment concentrations were used to establish the relevant endpoint for the study. The study met the relevant validity criteria according to the guideline (OECD, 203). No mortalities or sub-lethal effects were observed in the control, solvent control or any treatment group. The resulting LC_{50} was > 98.5 µg a.s./L (mean measured). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Anon. 1998b. Diflufenican: Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) under static conditions.

The acute toxicity of diflufenican to *O. mykiss* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under static conditions, at nominal treatment concentrations of 0 (control), 9.4, 18.8, 37.5, 75.0 and 150 µg a.s./L (a solvent control using DMF was also tested). The 150 µg a.s./L treatment group was above the limit of aqueous solubility. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore mean measured treatment concentrations were established at 7.3, 15.6, 28.2, 53.5 and 108.8 µg a.s./L (based on measurements at the test start, after 72 hours exposure and test termination), the mean measured treatment concentrations were used to establish the relevant endpoint for the study. The study met the relevant validity criteria according to the guideline (OECD, 203). No mortalities were observed in the control, or solvent control or any treatment group < 108.8 µg a.s./L, 10% mortality was observed at 108.8 µg a.s./L. The resulting LC_{50} was > 108.8 µg a.s./L (mean measured). Sub-lethal effects (including pigmentation disorders, accelerated respiration and lethargy) were observed in all treatment groups though only a few fish were affected in each case. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Anon. 2007a. Diflufenican technical: Acute toxicity to fish rainbow trout

The acute toxicity of diflufenican to *O. mykiss* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under semi-static conditions, at nominal concentrations of 0 (control) and 50 µg a.s./L (a solvent control was also tested) (the test solutions were renewed every 24 hours). It was noted that the nominal tested concentration was above the limit of solubility of diflufencian (44 µg a.s./L), however dosing was performed with a solvent and the treatment concetrations were confirmed. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore a mean measured treatment concentration was established at 32.8 µg a.s./L (based on measurements in the fresh (0 and 72 hours) and aged (24 and 96 hours) treatment solutions), the mean measured treatment concentration was used to establish the relevant endpoint for the study. The study met the relevant validity criteria according to the guideline (OECD, 203). No mortalities were observed in the control, solvent control or treatment group. Sub-lethal effects were observed in the treatment group, specifically darkened pigmentation of three fish. The resulting LC_{50} was > 32.8 µg a.s./L (mean measured). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Anon. 2007b. Diflufenican technical: Acute toxicity to fish

The acute toxicity of diflufenican to *Pimephales promelas* was assessed in a study performed to the guideline OECD 203 (1992) and according to GLP. Exposure to the test item was for 96 hours under semi-static conditions, at nominal concentrations of 0 (control) and 50 µg a.s./L (a solvent control was also tested) (the test solutions were renewed every 24 hours). It was noted that the nominal tested concentration was above the limit of solubility of diflufencian (44 µg a.s./L), however dosing was performed with a solvent and the treatment concetrations were confirmed. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore a mean measured treatment concentration was established at 39.8 µg a.s./L (based on measurements in the fresh (0 and 72 hours) and aged (24 and 96 hours) treatment solutions), the mean measured treatment concentration was used to establish the relevant endpoint for the study. The study met the relevant validity criteria according to the guideline (OECD, 203). No mortalities were observed in the control, solvent control or treatment group. Sub-lethal effects were observed in the treatment group, specifically darkened pigmentation of three fish. The resulting LC_{50} was > 39.8 µg a.s./L (mean measured). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Overall conclusion

There are sufficient suitable studies to allow classification of the acute hazard to fish from exposure to diflufenican. **The endpoint selected for use in hazard classification is LC_{50} > 98.5 µg a.s./L (Anon. 1998c)**, no mortalities were observed in any study and all endpoints are unbounded. Therefore use of the

lowest tested treatment concentration is not required to set the LC_{50}, and the higher endpoint has been selected to reflect the LC_{50}. It is noted that in all cases the maximum tested concentrations are above the limit of solubility of diflufenican (44 µg a.s./L), though all tests were performed with a solvent so increased exposure concentrations could be obtained, and all endpoints were based on measured concentrations. The selected endpoint was that set in the previous evaluation of the active substance (under Dir. 91/414/EEC), further consideration was not considered necessary as it is noted that ultimately fish are not the most acutely sensitive taxa and therefore not critical for setting the hazard classification.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Acute studies were provided both for the original approval for diflufenican under Dir. 91/414/EEC, and for the renewal of the active under Reg. (EC) 1107/2009 (circa 2018). Those studies considered acceptable are summarised below.

Odin-Feurtet (1999f). Diflufenican: Acute toxicity (48 hours) to daphnids (*Daphnia magna*) under static conditions.

The acute toxicity of diflufenican to *D. magna* was assessed in a study performed to the guideline OECD 202 (1984) and according to GLP. Exposure to the test item was for 48 hours under static conditions, at nominal concentrations of 0 (control), 0.03, 0.06, 0.13, 0.25 and 0.5 mg a.s./L (a solvent control was also tested). The 0.5 mg a.s./L treatment group was above the visual limit of solubility, as indicated by a precipitate in the test solution. The test item was not within ± 20 % of the nominal for all concentrations at all observation points during the study; therefore mean measured treatment concentrations were established at 0.03, 0.05, 0.09, 0.15 and 0.24 mg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentration was used to establish the relevant endpoint for the study. The study met the relevant validity criteria according to the guideline (OECD, 202). No immobilisation or other sub-lethal effects were observed in the control groups or treated groups. The resulting EC_{50} was > 0.24 mg a.s./L (mean measured). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Wilby (2007e). Diflufenican technical: Acute toxicity to *Daphnia Magna*.

The acute toxicity of diflufenican to *D. magna* was assessed in a study performed to the guideline OECD 202 (2004) and according to GLP. Exposure to the test item was for 48 hours under static conditions, at nominal concentrations of 0 (control) and 50 µg a.s./L (a solvent control was also tested). The test item was not maintained within ± 20 % of the nominal during the study; therefore a mean measured treatment concentration was established at 42 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentration was used to establish the relevant endpoint for the study. The study met the relevant validity criteria according to the guideline (OECD, 202). No immobilisation or other sub-lethal effects were observed in the control groups or treatment group. The resulting EC_{50} was > 42 µg a.s./L (mean measured). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Overall conclusion

There are sufficient suitable studies to allow classification of the acute hazard to aquatic invertebrates from exposure to diflufenican. **The endpoint selected for use in hazard classification is EC_{50} > 240 µg a.s./L (Odin-Feurtet, 1999f)**, no immobilisation was observed in any study and all endpoints are unbounded. Therefore use of the lowest tested treatment concentration is not supported to set the EC_{50}, and the higher endpoint has been selected to reflect the EC_{50}. It is noted that in all cases the maximum tested concentrations are above the limit of solubility of diflufenican (44 µg a.s./L), though all tests were performed with a solvent so increased exposure concentrations could be obtained, and all endpoints were based on measured concentrations. It is noted that ultimately aquatic invertebrates are not the most acutely sensitive taxa and therefore not critical for setting the hazard classification.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Acute studies were provided both for the original approval for diflufenican under Dir. 91/414/EEC, and for the renewal of the active under Reg. (EC) 1107/2009 (circa 2018). Those studies considered acceptable are summarised below.

Algae:

Wilby (2007g). Diflufenican technical: Algal growth inhibition assay.

The toxicity of diflufenican to *Raphidocelis subcapitata* (formerly *P. subcapitata*, as referred to in the report, and *S. capricornutum*) was assessed in a study performed to the guideline OECD 201 (2006) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control) 0.0427, 0.0939, 0.207, 0.455 and 1.0 µg a.s./L (a solvent control was also tested). The test item was not maintained within ± 20 % of the nominal during the study; therefore mean measured treatment concentrations were established at 0.0559, 0.0953, 0.125, 0.451 and 1.10 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

There was a significant difference in the cell density between the control and solvent control groups (18 % fewer cells/mL in the solvent control compared to the negative control), and therefore the treatment groups were compared to the solvent control. Biomass (based on area under the growth curve) was inhibited compared to the solvent control by -2.9 – 94.5 % after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was -0.3 – 71.7 % compared to the solvent control. The resulting E_rC_{50} = 0.6 µg a.s./L and E_rC_{10} = 0.157 µg a.s./L, the NOE_rC is 0.0953 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

(It is noted that a 'recovery phase' was also established after the standard exposure phase, in which algal cells were transferred to fresh, untreated, test solution at the end of the exposure period and then monitored for recovery potential. Though as this does not impact the exposure phase or consideration of the hazard further details of the results related to recovery have not been included here.)

Wilby (2007h). Diflufenican technical: Algal growth inhibition assay *Anabaena* spp.

The toxicity of diflufenican to *Anabaena* sp. was assessed in a study performed to the guideline OECD 201 (2006) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control) 0.164, 0.41, 1.02, 2.56, 6.4, 16 and 40 µg a.s./L (a solvent control was also tested). The test item was not maintained within ± 20 % of the nominal during the study; therefore mean measured treatment concentrations were established at 0.124, 0.370, 1.40, 4.02, 8.10, 26.0 and 43.7 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201) regarding the increase in cell density (≥ 16 fold increase by 72 hours) and the

coefficient of variation (CoV) over the whole test period (CoV < 7%). However, the CoV of the mean section by section growth rate did not meet the relevant criterion (required CoV ≤ 35 %; observed CoV = 39.9 %). It is noted that *Anabaena* sp. forms cell aggregations that can lead to greater variability in the measured cell densities, given this and the small deviation from the required CoV criterion it was considered acceptable to consider the results of this study further.

Biomass (based on area under the growth curve) was inhibited compared to the solvent control by -1.5 – 51.8 % after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was 0.5 – 18.4 % compared to the solvent control. The resulting E_rC_{50} > 43.7 µg a.s./L and E_rC_{10} = 16.05 µg a.s./L, the NOE_rC is 0.37 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Hoberg (1997a). Diflufenican technical - Toxicity to the freshwater diatom, *Navicula pelliculosa*.

The toxicity of diflufenican to *N. pelliculosa* was assessed in a study performed to the guideline OECD 201 (1984) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control) 0.04, 0.10, 0.26, 0.64, 1.60, 4.00 and 10 µg a.s./L (a solvent control was also tested). Mean measured treatment concentrations were established at 0.042, 0.098, 0.28, 0.65, 1.4, 3.8 and 9.5 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

Biomass was statistically significantly inhibited compared to the solvent control at concentrations ≥ 0.28 µg a.s./L after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was statistically significant at concentrations ≥ 0.65 µg a.s./L. The resulting E_rC_{50} = 4.3 µg a.s./L, the NOE_rC is 0.28 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Wilby (2007i). Diflufenican technical: Algal growth inhibition assay *Navicula pelliculosa*.

The toxicity of diflufenican to *N. pelliculosa* was assessed in a study performed to the guideline OECD 201 (2006) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control), 0.427, 0.939, 2.07, 4.55 and 10 µg a.s./L (a solvent control was also tested). The test item was not maintained within ± 20 % of the nominal during the study; therefore mean measured treatment concentrations were established at 0.364, 0.895, 1.96, 4.91 and 10.9 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

Biomass (based on area under the growth curve) was inhibited compared to the solvent control by -1.4 – 100 % after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was 0 – 87.2 % compared to the solvent control. The resulting E_rC_{50} = 6.08 µg a.s./L and E_rC_{10} = 2.14 µg a.s./L, the NOE_rC is 0.895 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Hoberg (1998f). Diflufenican - Toxicity to the freshwater blue-green alga, *Microcystis aeruginosa*.

The toxicity of diflufenican to *M. aeruginosa* was assessed in a study performed to the guideline OECD 201 (1984) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control), 0.41, 1.0, 2.6, 6.4, 16, 40 and 100 µg a.s./L (a solvent control was also tested). Mean measured treatment concentrations were established at 0.39, 0. 95, 3.0, 7.0, 15, 43 and 98 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

The resulting E_rC_{50} is > 98 µg a.s./L, the NOE_rC is 7.0 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Juckeland (2015a). Effects of diflufenican technical on *Chlamydomonas rheinhardtii* in an algal growth inhibition test with a recovery phase.

The toxicity of diflufenican to *C. rheinhardtii* was assessed in a study performed to the guideline OECD 201 (2006) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control), 5.40, 11.3, 23.7, 50.0 and 105.0 µg a.s./L (a solvent control was also tested). The test item was not maintained within ± 20 % of the nominal during the study; therefore mean measured treatment concentrations were established at 4.77, 9.38, 18.1, 38.1 and 64.3 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

Biomass (based on yield) was inhibited compared to the control by 0.8 – 95.9 % after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was 0.2 – 64.7 % compared to the control. The resulting E_rC_{50} = 44.9 µg a.s./L and E_rC_{10} = 10.0 µg a.s./L, the NOE_rC is 4.77 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

(It is noted that a 'recovery phase' was also established after the standard exposure phase, in which algal cells were transferred to fresh, untreated, test solution at the end of the exposure period and then monitored for recovery potential. Though as this does not impact the exposure phase or consideration of the hazard further details of the results related to recovery have not been included here.)

Juckeland (2015b). Effects of diflufenican technical on *Chlorella vulgaris* in an algal growth inhibition test with a recovery phase.

The toxicity of diflufenican to *C. vulgaris* was assessed in a study performed to the guideline OECD 201 (2006) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control), 20.7, 31.1, 46.7, 70.0 and 105.0 µg a.s./L (a solvent control was also tested). The test item was maintained within ± 20 % of the nominal during the study, therefore nominal treatment concentration were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

Biomass (based on yield) was inhibited compared to the solvent control by 1.0 – 76.6 % after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was 0.2 – 32.0 % compared to the control. The resulting E_rC_{50} = 139.5 µg a.s./L (based on extrapolation outside the range of tested treatment concentrations) and E_rC_{10} = 64.5 µg a.s./L, the NOE_rC is 31.1 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

(It is noted that a 'recovery phase' was also established after the standard exposure phase, in which algal cells were transferred to fresh, untreated, test solution at the end of the exposure period and then monitored for recovery potential. Though as this does not impact the exposure phase or consideration of the hazard further details of the results related to recovery have not been included here.)

Juckeland (2015c). Effects of diflufenican technical on *Desmodesmus communis* in an algal growth inhibition test with a recovery phase.

The toxicity of diflufenican to *D. communis* was assessed in a study performed to the guideline OECD 201 (2006) and according to GLP. Exposure to the test item was for 72 hours under static conditions, at nominal concentrations of 0 (control), 0.05, 0.13, 0.33, 0.85, 2.22, 5.76 and 15.0 µg a.s./L (a solvent control was also tested). The test item was not maintained within ± 20 % of the nominal during the study; therefore mean

measured treatment concentrations were established at 0.0457, 0.110, 0.319, 0.426, 2.44, 4.48 and 13.3 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 201).

Biomass (based on yield) was inhibited compared to the control by 1.6 – 76.3 % after 72 hours exposure in the treatment groups. The inhibition of growth rate, in the treatment groups, was 0.52 – 31.27 % compared to the control. The resulting E_rC_{50} = 21.5 µg a.s./L (based on extrapolation outside the range of tested treatment concentrations) and E_rC_{10} = 1.08 µg a.s./L, the NOE_rC is 0.43 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

(It is noted that a 'recovery phase' was also established after the standard exposure phase, in which algal cells were transferred to fresh, untreated, test solution at the end of the exposure period and then monitored for recovery potential. Though as this does not impact the exposure phase or consideration of the hazard further details of the results related to recovery have not been included here.)

Other Aquatic plants:

Hoberg (1998h). Diflufenican - Toxicity to the duckweed, *Lemna gibba*.

The toxicity of diflufenican to *L. gibba* was assessed in a study performed to the US EPA FIFRA guidelines 12.-2 and 123-2, and according to GLP. The studies performance was also considered in relation to the OECD 221 (2006) guidance, and it also met the validity criteria under that guideline. Exposure to the test item was for 14 days under semi-static conditions (fresh solutions on days 0, 3, 6, 9 and 12), at nominal concentrations of 0 (control), 0.41, 1.0, 2.6, 6.4, 16, 40 and 100 µg a.s./L (a solvent control was also tested). Mean measured treatment concentrations were established at 0.46, 1.1, 2.8, 6.7, 15, 39 and 95 µg a.s./L. However, treatment concentrations were only measured on days 0 (fresh solutions) and 3 (aged solutions), no other treatment solutions (fresh or aged) were analysed to confirm the treatment concentrations. Whilst this introduces additional uncertainty regarding the true treatment concentration, it was considered that 1) the period between renewals of the test solution was 3 days, and therefore identical to the time frame over which the available measurements were performed; and 2) the DT_{50} of diflufenican in water according to the renewal report under Reg. (EC) 1107/2009 was 31.7 days in water, and so the test item should be reasonably stable over the renewal period used in the study (3 days). Given these observations it was considered that the sampling was sufficient to justify using the nominal concentrations to establish an E_rC_{50}, whilst noting there is uncertainty due to the limitations of the analytical results.

The inhibition of growth rate based on frond numbers, in the treatment groups, was -2.6 – 54.5 % compared to the pooled control. The resulting E_rC_{50} is > 40 µg a.s./L, the NOE_rC is 16 µg a.s./L (based on growth rate). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Wilby (2007k). Diflufenican technical: Higher plant (*Lemna minor*) growth inhibition test.

The toxicity of diflufenican to *L. minor* was assessed in a study performed to the guideline OECD 221 (2006), and according to GLP. Exposure to the test item was for 7 days under semi-static conditions (fresh solutions on days 0, 3 and 5), at nominal concentrations of 0 (control), 1.02, 2.56, 6.4, 16 and 40 µg a.s./L (a solvent control was also tested).

Analytical confirmation of the treatment concentrations was performed and the results are summarised in Table . However, for several samples there were 'variable results' according to the Study Author and the analysis was rerun, this adds uncertainty regarding the true exposure concentrations. All the initial measured concentrations (in fresh solutions, on days 0, 3 and 5) were within ± 20 % of nominal with the exception of two samples. The aged samples do not indicate a decline of the test item to less than 20 % of the nominal (baring the reanalysis of the 16 µg a.s./L treatment; see Table). Additionally it is noted that the DT_{50} of diflufenican is 31.7 days (as established in the evaluation of diflufenican under Reg. (EC) 1107/2009, circa 2018), which supports the conclusion that treatment concentrations are likely to have been maintained. Given these observations it was concluded that the results should be considered further, but that they could be based

on nominal treatment concentrations rather than mean measured concentrations given the uncertainty regarding the analytical methods.

Table 18: Inhibition of growth rate for each of the measured parameters

Nominal concentration (µg a.s./L)	Measured concentrations											
	Day 0 (Fresh)		Day 3 (aged)		Day 3 (fresh)		Day 5 (aged)		Day 5 (fresh)		Day 7 (aged)	
	µg a.s./L	% nom.	µg a.s./L	% nom.	µg a.s./L	% nom.	µg a.s./L	% nom.	µg a.s./L	% nom.	µg a.s./L	% nom.
Control	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
Solvent control	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-	n.d.	-
1.02	1.25	123	2.13 **1.49**	209 **146**	0.963	94	1.26 **1.43**	124 **140**	1.08	106	1.08	106
2.56	3.44 **2.80**	134 **109**	4.53 **4.00**	177 **156**	2.35	92	2.80	109	2.81	110	3.41 **2.81**	133 **110**
6.4	6.60	103	8.85 **8.12**	138 **127**	6.97	109	7.05	110	6.37	100	6.71	105
6.4A	-	-	7.67 **6.72**	120 **105**	-	-	7.23	113	-	-	5.59	87
16	18.0	113	18.2 **12.2**	114 **76**	16.7	104	19.0	119	19.1	119	17.4	109
40	46.1	115	58.8 **40.1**	147 **100**	57.3 **39.6**	143 **99**	46.5	116	39.1	98	40.1	100

n.d. = Not determined; % nom. = Percentage of nominal concentration; A = culture medium incubated under test conditions without plants; bold value is analysis of reserve sample due to variable results; - = not tested / not calculated

The parameters frond numbers and dry weight were measured to investigate the impact of the test item, additionally observations of phytotoxicity were reported. Less than 50 % effects on growth rate in relation to dry weight were reported in the study. Based on the raw frond numbers less than 50 % effects on growth rate were also reported for all treatment groups, however large numbers of fronds were observed to be chlorotic or necrotic at the highest treatment concentration (40 µg a.s./L, nominal). When the numbers of chlorotic/necrotic fronds were excluded from the total number of fronds reported at 40 µg a.s./L (nominal), the results indicate about a 48 % inhibition of the growth rate. It was concluded that the phytotoxic symptoms should be taken into account and that therefore there was about a 48 % inhibition of growth rate at the maximum treatment concentration, given the uncertainty over the analytical results the E_rC_{50} was set at 40 µg a.s./L (nominal) to account for these observations, and the NOEC based on growth rate was concluded to be 16 µg a.s./L (nominal). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Hermes & Emmet (2016). Diflufenican technical: Toxicity to the Aquatic Plant *Myriophyllum spicatum* in a Static Growth Inhibition Test with a Prior Rooting Phase.

The toxicity of diflufenican to *M. spicatum* was assessed in a study performed to the guideline OECD 239 (2014), and according to GLP. Exposure to the test item was for 14 days under static conditions, at nominal concentrations of 0 (control), 2.00, 6.34, 20.0, 63.3 and 200 µg a.s./L (a solvent control was also tested). This test was performed in a water-sediment test system. The test item concentrations were not maintained within ± 20 % of the nominal during the test; therefore, mean measured treatment concentrations were established at

1.02, 4.04, 12.5, 38.3, 107 and 116 µg a.s./L in the overlying test water. The study met all the relevant validity criteria according to the guideline (OECD 239).

The inhibition of growth rate, relative to the control, for each of the measured parameters is summarised in Table . The most sensitive parameter was effects on dry weight (Table), but in all cases there was a shallow dose response curve at ≥ 4.04 µg a.s./L. The report established an $E_rC_{50,\ dry\ weight}$ of 19.8 µg a.s./L which exceeds the lowest concentration at which > 50 % inhibition was observed. Visual interpretation of the fitted model indicates a reasonable fit given the nature of the data. However, given the shallow dose response relationship at ≥ 4.04 µg a.s./L, and wide between replicate variation at lower treatment concentrations, there is some uncertainty regarding the accuracy of the reported $E_rC_{50,\ dry\ weight}$. It is therefore proposed to set the $E_rC_{50,\ dry\ weight}$ at the maximum concentration at which < 50 % inhibition was observed in relation to dry weight (> 4.04 µg a.s./L; see Table 32).

Table 19: Inhibition of growth rate for each of the measured parameters

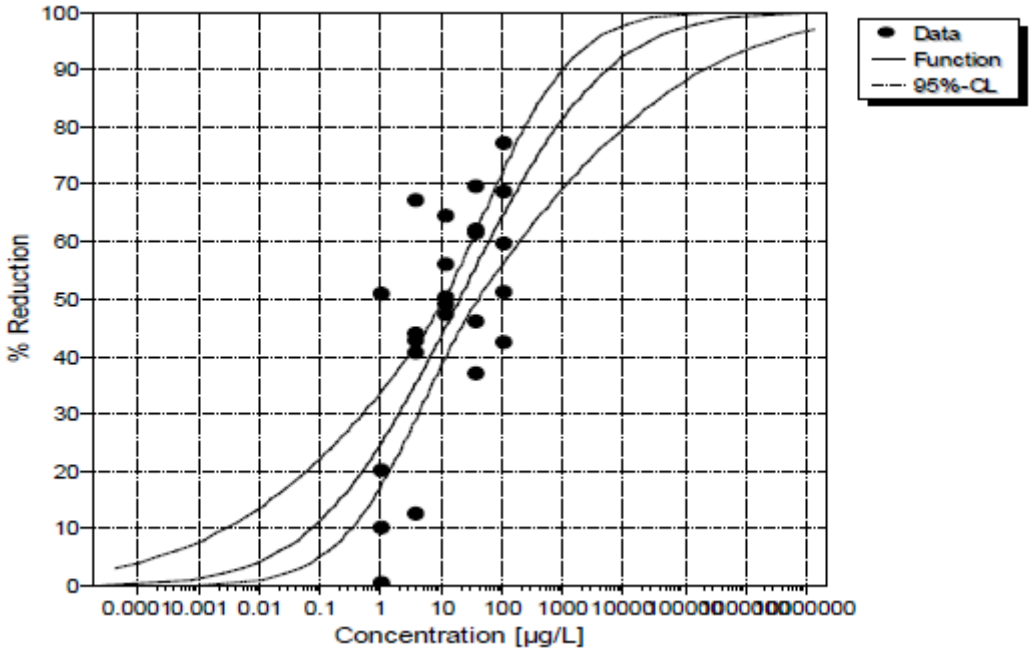
Geometric mean test concentration (µg a.s./L)	% inhibition of growth rate		
	Based on dry weight	Based on fresh weight	Based on total shoot length
Control	n.a.	n.a.	n.a.
Solvent control	-0.2	-3.1	-1.2
1.02	15.4	-0.5	8.2
4.04	41.3 *	15.5 *	34.6 *
12.5	53.3 *	36.3 *	31.5 *
38.3	55.1 *	43.3 *	31.2 *
107	59.7 *	50.1 *	30.0 *

* Statistically significantly different to the control

Sub-lethal effects were also reported in all treatment groups > 1.02 µg a.s./L (including few roots, shortened roots, chlorosis, and shortened shoot tops at day 14).

The resulting $E_rC_{50,\ dry\ weight}$ from the study is > 4.04 µg a.s./L. A NOEC, based on inhibition of growth rate, could be established at 1.02 µg a.s./L, however it is noted that a 15.4 % inhibition of the growth rate (based on dry weight) was observed at that treatment concentration. An E_rC_{10} could only be established via extrapolation below the lowest treatment concentration used in the study ($E_rC_{10,\ dry\ weight}$ = 0.073 µg a.s./L; 95% C.I. 0.003 – 0.330; see Figure 01 for the fitted model used to estimate the $E_rC_{10,\ dry\ weight}$). Whilst the fitted model (see Figure 1) is a reasonable approximation given the available data, there is a wide spread of responses particularly at lower treatment concentrations. The resulting wide confidence intervals around the proposed E_rC_{10} (100 fold difference between the minimum and maximum values; 0.003 – 0.330) indicate uncertainty regarding the true value. Additionally the E_rC_{10} falls outside the range of measured treatment concentrations and the ability of the data to inform the fit of the model. Therefore, the E_rC_{10} was not recommended for use in hazard classification. Though the study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Figure 1: Concentration effect curve fitted to dry weight data, and used to establish the E_rC_{10} estimate



Juckeland (2015d). Effects of diflufenican technical on *Myriophyllum spicatum* in a water-sediment system.

The toxicity of diflufenican to *M. spicatum* was assessed in a study performed to the guideline OECD 239 (2014) and according to GLP. Exposure to the test item was for 14 days under static conditions, at nominal concentrations of 0 (control), 1.03, 3.09, 9.26, 27.8, 83.4 and 250.0 µg a.s./L (a solvent control was also tested). The test was conducted in a water-sediment test system. The test item was not maintained within ± 20 % of the nominal during the study, in the overlying water; therefore mean measured treatment concentrations were established at 1.06, 3.46, 8.09, 19.9, 42.2 and 118.5 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 239).

The following parameters were measured to investigate the effects of the test item, dry weight, fresh weight, total shoot length, main shoot length, and number of lateral branches. The most sensitive parameters in terms of effects on growth rate were the dry weight and total shoot length. The worst case $E_rC_{50,\ dry\ weight}$ = 14.8 µg a.s./L which was considered suitable for use in risk assessment. Though it is noted that an R^2 value of 0.603 was reported for the relevant E_rC_{50} estimate and hence there is uncertainty regarding the accuracy of the E_rC_{50}; visual estimation indicates a reasonable fit of the model to the data, given the underlying variance, and hence despite the uncertainty the endpoint is considered suitable for further consideration. The NOEC (based on on growth rate and dry weight) was 1.06 µg a.s./L and the $E_rC_{10,\ dry\ weight}$ was 0.77 µg a.s./L. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Kuhl (2016a). Toxicity of diflufenican (AE F088657) to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test - Final report.

The toxicity of diflufenican to *M. spicatum* was assessed in a study performed to the guideline OECD 239 (2014) and according to GLP. Exposure to the test item was for 14 days under static conditions, at nominal concentrations of 0 (control), 1.43, 4.58, 14.6, 46.9 and 150 µg a.s./L (a solvent control was also tested). The test was conducted in a water-sediment test system. The test item was not maintained within ± 20 % of the

nominal during the study, in the overlying water; therefore mean measured treatment concentrations were established at 1.24, 3.77, 12.5, 41.6 and 101 µg a.s./L (based on measurements at test initiation and termination), the mean measured treatment concentrations were used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the guideline (OECD, 239).

The following parameters were measured to investigate the effects of the test item, dry weight, fresh weight, and total shoot length. Less than 50 % inhibition was observed for all measured parameters in terms of growth rate, so E_rC_{50} estimates can only be concluded to be > 101 µg a.s./L, the maximum tested treatment concentration. A NOE_rC was established at 3.77 µg a.s./L, based on fresh weight, neither of the other parameters resulted in any statistically significant effects. E_rC_{10} values based on both dry and fresh weight were established (5.28 and 23.0 µg a.s./L respectively) though no confidence intervals for these estimates are available. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Overall conclusion

There are sufficient suitable studies to allow classification of the acute hazard to algae and other aquatic plants from exposure to diflufenican. **The endpoint selected for use in hazard classification is E_rC_{50} = 0.6 µg a.s./L (Wilby, 2007g)**, this is based on the most sensitive taxon (*R. subcapitata*) considered in the available valid studies. Algae are the group of organisms most acutely sensitive to exposure to diflufenican based on the available information.

(It is noted that *R. subcapitata* may not represent the most sensitive algal species. Within the dossier submitted to support diflufenican under Reg. (EC) 1107/2009 a study was available testing *Ankistrodesmus falcatus* that exhibited an E_rC_{50} of 0.064 µg a.s./L, approximately 10 fold lower than that for *R. subcapitata*. The study was not considered valid due to failure to meet the validity criterion related to the mean coefficient of variation for section-by-section growth rates specified in the OECD 201 guideline. No other studies are available for this species. *A. falcatus* is a novel test species not normally used within the OECD 201 test guideline and it was noted in the study report that there appeared to be a lag phase to the development of the cultures which may have explained the failure to meet the validity criterion. However, the study was not considered valid and so has not been used in hazard classification, but it is highlighted that this information suggests that other test species may exhibit greater sensitivity to diflufenican than *R. subcapitata*.)

11.5.4 Acute (short-term) toxicity to other aquatic organisms

Acute studies were provided for the renewal of the active (circa 2018). The study considered acceptable is summarised below.

Anon. (2013). Acute toxicity of diflufenican technical to the African clawed frog (*Xenopus laevis*) under static conditions.

The toxicity of diflufenican to tadpoles of *X. laevis* was assessed, no specific guideline was available for this species however the test protocol was based on the guidelines OECD 203 and US EPA 850.1075. The study was conducted according to GLP. Exposure to the test item was for 48 hours under static conditions, at nominal concentrations of 0 (control) and 120 µg a.s./L (a solvent control was also tested). The initial test concentration as not within ± 20 % of the nominal, however the measured concentration was maintained during the study. A mean measured treatment concentration was established at 70.5 µg a.s./L, the mean measured treatment concentration was used to establish the relevant endpoints for the study. The study met the relevant validity criteria according to the OECD 203 guideline, though the criteria were not set for consideration of *X. laevis*.

A single mortality was observed in each of the control, solvent control and treatment group after 48 hours (out of an initial cohort of 30 tadpoles). Therefore, the LC_{50} is > 70.5 µg a.s./L and the NOEC is 70.5 µg a.s./L. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

11.6 Chronic (long-term) aquatic hazard

Studies available during the renewal of diflufenican as an active substance, circa 2018, under Reg. (EC) 1107/2009 are summarised in Table . All the listed studies have been conducted according to GLP. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes. Only studies submitted testing the technical substance, diflufenican, have been summarised below. Studies not deemed suitable for use in risk assessment under Reg. (EC) 1107/2009 have not been included, or discussed further, as they are also not considered suitable for use in hazard classification under Reg. (EC) 1272/2008.

Note: Studies with sediment dwelling invertebrates, tested in water sediment systems dosed via the sediment, have not been included in Table 20. The relevance of tests conducted where the test item is added via the sediment (i.e. sediment-spiked) is unclear in the context of Reg. (EC) 1272/2008, and therefore these data points have not been reproduced below. Water-sediment studies where the systems were dosed via the water phase (i.e. water-spiked) and endpoints which were based on measured levels in the overlying water (those for Chironomus and Myriophyllum) have been included however, as their endpoint may be considered suitable for hazard classification purposes.

Table 20: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Reference	Submitted for original approval (O) or renewal (N)
Fish					
US EPA FIFRA 72-4 and OTTPS 850.1400; 35-days, static, early-life-stage study	*P. promelas*	diflufenican (purity 96.8 %)	EC_{10} = n.a. NOEC = 15 µg /L (m.m.)	Anon. 1998d	O
OECD 210; 28-days, flow through, early-life-stage study	***P. promelas***	**diflufenican (purity 98.8 %)**	**EC_{10} = 5.43 µg /L (m.m.)** NOEC = 3.05 µg /L (m.m.)	**Anon. 2007c**	**N**
OECD draft guideline (Nov. 1994); 28-days, flow through, juvenile growth study	*O. mykiss*	diflufenican (purity 96.7 %)	EC_{10} = n.a. NOEC = 19.2 µg /L (m.m.)	Anon. 1997	O
Aquatic invertebrates					
OECD 211; 21-days, static renewal	*D. magna*	diflufenican (purity 96.8 %)	EC_{10} = n.a. NOEC = 52 µg /L (m.m.)	Putt (2000)	O
OECD 211; 21-days, static renewal	*D. magna*	diflufenican (purity 98.8 %)	EC_{10} = n.a. NOEC = 22.2 µg /L (g.m.)	Burke (2007b)	N
Sediment dwelling invertebrates					
BBA guideline (1995) [2]; 28-days, static, water-sediment system, spiked water	***Chironomus riparius***	**diflufenican (purity 96.7 %)**	EC_{10} = n.a. **NOEC = 12.4 µg /L (m.m.)[3]**	**McElligott (1996b)**	**O**
Algae					
OECD 201; 72-hours, static exposure	***Raphidocelis subcapitata* (Formerly *P.***	**diflufenican (purity 98.8 %)**	**E_rC_{10} = 0.157 µg /L (g.m.)** NOE_rC = 0.095 µg /L	**Wilby (2007g)**	**N**

	***subcapitata* and *S. capricornatum*)**		(g.m.)		
OECD 201; 72-hours, static exposure	*Anabaena sp.*	diflufenican (purity 98.8 %)	E_rC_{10} = 16.05 µg /L (g.m.) NOE_rC = 0.37 µg /L (g.m.)	Wilby (2007h)	N
OECD 201; 72-hours, static exposure	*Navicula pelliculosa*	diflufenican (purity 96.7 %)	E_rC_{10} = n.a. NOE_rC = 0.28 µg /L (m.m.)	Hoberg (1997a)	O
OECD 201; 72-hours, static exposure	*N. pelliculosa*	diflufenican (purity 98.8 %)	E_rC_{10} = 2.14 µg /L (g.m.) NOE_rC = 0.895 µg /L (g.m.)	Wilby (2007i)	N
OECD 201; 72-hours, static exposure	*Mycrocystis aeruginosa*	diflufenican (purity 96.8 %)	E_rC_{10} = n.a. NOE_rC = 7 µg /L (m.m.)	Hoberg (1998f)	O
OECD 201; 72-hours, static exposure	*Chlamydomonas rheinhardtii*	diflufenican (purity 100 ± 0.6 %)	E_rC_{10} = 10.0 µg /L (g.m.) NOE_rC = 4.77 µg /L (g.m.)	Juckeland (2015a)	N
OECD 201; 72-hours, static exposure	*Chorella vulgaris*	diflufenican (purity 100 ± 0.6 %)	E_rC_{10} = 64.5 µg /L (nom.) NOE_rC = 31.1 (nom.)	Juckeland (2015b)	N
OECD 201; 72-hours, static exposure	*Desmodesmus communis*	diflufenican (purity 100 ± 0.6 %)	E_rC_{10} = 1.08 µg /L (g.m.) NOE_rC = 0.43 µg /L (g.m.)	Juckeland (2015c)	N
Other aquatic plants					
US EPA FIFRA Guidelines 12-2 and 123-2; 14-days, static exposure	*Lemna gibba*	diflufenican (purity 96.8 %)	E_rC_{10} = n.a. NOE_rC = 16 µg /L (nom.)	Hoberg (1998h)	O
OECD 221; 7-days, semi static exposure	*Lemna minor*	diflufenican (purity 98.8 %)	E_rC_{10} = n.a. NOE_rC = 16 µg /L (nom.)	Wilby (2007k)	N
OECD 239; 14-days, static exposure	*Myriophyllum spicatum*	diflufenican (purity 98.8 %)	E_rC_{10} = n.a.[4] NOE_rC = 1.02 µg /L (g.m.)	Hermes & Emmet (2016)	N
OECD 239; 14-days, static exposure	*M. spicatum*	diflufenican (purity 100 ± 0.6 %)	E_rC_{10} = 0.77 µg /L (g.m.) NOE_rC = 1.06 µg /L (g.m.)	Juckeland (2015d)	N
OECD 239; 14-days, static exposure	*M. spicatum*	diflufenican (purity 97.3 %)	E_rC_{10} = 5.28 µg /L (g.m.) NOE_rC = 3.77 µg /L (g.m.)	Kuhl (2016a)	N

Bold entries are the endpoints considered most suitable to set the hazard classification for the active substance for each group of organisms.

Note: Data owners have been identified in square brackets for all new studies, new in the context of the most recent renewal of diflufenican under Reg. (EC) 1107/2009, circa 2018.

[1] m.m. = measured concentration; g.m.= geometric mean measured concentration; nom. = nominal concentration

[2] BBA guideline (1995): Effects of plant protection products on the development of sediment dwelling larvae of *C. riparius* in a water sediment system.

[3] This endpoint has been altered during assessment to reflect the dissipation of the test item from the overlying water of the test system; it reflects the geometric mean measured concentration in the water phase, as opposed to the nominal.

[4] EC_{10} estimates included in the study report were extrapolated below the lowest treatment group tested in the study.

11.6.1 Chronic toxicity to fish

Long-term studies were provided both for the original approval for diflufenican under Dir. 91/414/EEC, and for the renewal of the active under Reg. (EC) 1107/2009 (circa 2018). Those studies considered acceptable are summarised below.

Anon. 1998d. Diflufenican - Early life-stage toxicity test with fathead minnow (*Pimephales promelas*).

The chronic toxicity of diflufenican to early life stage *P. promelas* was assessed in a study performed to the guideline US EAP FIFRA 72-4 (OPPTS 850.1400, 1996) and according to GLP. Exposure to the test item was for 35 days under flow-through conditions, at nominal concentrations of 0 (control), 0.62, 1.9. 5.6, 17 and 50 µg a.s./L (a solvent control was also tested). At test initiation 60 embryos (≤ 24 hours old), per replicate (two replicates per treatment), were placed in incubation cups. Dead/live embryos were counted daily until hatching was complete (day 4) then surviving larvae were thinned to 40 per replicate and transferred to aquaria for the remainder of the study.

Treatment concentrations were measured on days 0, 4, 10, 17, 24, 31 and 35. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore arithmetic mean measured treatment concentrations were established at 0.69, 1.7, 5.1, 15 and 47 µg a.s./L, and the mean measured treatment concentrations were used to establish the relevant endpoints for the study. It is noted that the quality control samples indicted a positive bias on day 4 (in the 0.69 µg a.s./L treatment group) and therefore the measured recoveries were adjusted to account for this observation. This approach was considered acceptable during evaluation, based on consideration by a chemistry specialist and the observation that if the day 4 results were excluded there would be a negligible effect on the mean measured treatment concentration (0.64 vs. 0.69 µg a.s./L).

The study met the relevant validity criteria according to the guidelines (which are the same as under OECD 210). No effect on larval survival, pre or post hatch, was reported for any treatment group. Statistically significant effects on growth (in terms of total length, wet weight and dry weight) were observed at 1.7, 5.1 and 47 µg a.s./L, no significant effects were observed at 15 µg a.s./L (see Table). Although effects compared to the pooled controls were determined to be statistically significant at 1.7 and 5.1 µg a.s./L, these were considered to be relatively slight (close to or within 10%) and there were also no significant effects reported at 15 µg a.s./L, so there is a lack of a clear dose response relationship at ≤ 15 µg a.s./L. The NOEC for the study was therefore concluded to be 15 µg a.s./L (based on total length, wet weight and dry weight).

Table 21: Reported results related to growth

Mean measured concentration (µg a.s./L)	Mean total length (mm) [a]	Mean wet weight (g) [a]	Mean dry weight (g) [a]
Control	32.7	0.36	0.087
Solvent control	32.4	0.36	0.088
Pooled control	32.6	0.36	0.087
0.69	32.1 (1.5)	0.34 (5.6)	0.082 (5.7)
1.7	31.5 * (3.4)	0.31 * (14)	0.076 * (13)
5.1	31.5 * (3.4)	0.32 * (11)	0.077 (11)
15	31.9 (2.1)	0.33 (8.3)	0.080 (8.0)
47	30.0 * (8.0)	0.27 * (25)	0.066 * (24)

* Statistically significantly reduced compared to the pooled control (Bonferroni's t-Test)

[a] Percentage inhibition (%) compared to the pooled control reported in parentheses (to 2 significant figures).

The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Anon. 2007c. Diflufenican technical: Fish early life stage toxicity test for fathead minnow.

The chronic toxicity of diflufenican to early life stage *P. promelas* was assessed in a study performed to the guideline US EAP OPPTS 850.1400 and OECD (210); the study was performed in accordance with GLP. Exposure to the test item was under flow-through conditions, at nominal concentrations of 0 (control), 1.28, 3.2, 8, 20 and 50 µg a.s./L (a solvent control was also tested). At test initiation 30 eggs (≤ 24 hours old), per replicate (two replicates per treatment), were placed in incubation cups. Dead/live embryos were counted daily until hatching was complete (day 6) then surviving larvae were transferred to aquaria for the remainder of the exposure phase (an additional 28 days).

Treatment concentrations were measured on days 0 and 1 pre-hatching and then on days 0, 7, 14, 21 and 28 during the post hatching phase. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore arithmetic mean measured treatment concentrations were established at 1.28, 3.05, 7.27, 20.3 and 45.4 µg a.s./L, the mean measured treatment concentrations were used to establish the relevant endpoints for the study.

Hatching success was 80 – 97 % of the initial cohort (60 individuals per replicate) in all treatment groups and controls apart from the 1.28 µg a.s./L treatment group. The 1.28 µg a.s./L treatment group exhibited a 55 % hatching success, however this was primarily due to low hatching success in one of the two treatment replicates (33 and 77 % hatching success in the two replicates). Larval survival was statistically significantly reduced compared to the solvent control at test termination in only the 45.4 µg a.s./L treatment group (84 % survival compared to 98 % survival). Statistically significant differences compared to the solvent control were reported in terms of length at ≥ 7.27 µg a.s./L and in terms of dry weight at ≥ 20.3 µg a.s./L. The resulting overall NOEC from the study was therefore based on length and set at 3.05 µg a.s./L. The lowest EC_{10} was 5.43 µg a.s./L (based on length, as established in an additional report).

The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Anon.1997. Diflufenican: Fish, juvenile growth test 28 days under flow-through conditions.

The chronic toxicity of diflufenican to juvenile *O. mykiss* (weight 1 – 5 g) was assessed in a study performed to the draft OECD guideline ‘proposal for fish juvenile growth test 28 days’ (Nov, 1994); the study was performed in accordance with GLP. Exposure to the test item was under flow-through conditions, at nominal concentrations of 0 (control), 3, 8, 20, 40 and 80 µg a.s./L.

The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore arithmetic mean measured treatment concentrations were established at 2.3, 6.4, 12.5, 19.2 and 43 µg a.s./L, the mean measured treatment concentrations were used to establish the relevant endpoints for the study.

After 28 days, mortality had only been observed in a single treatment group. Two dead fish were reported in the 19.2 µg a.s./L treatment (12.5 %), which was attributed to a technical issue in the study report (specific details not reported). No other mortality was observed, even at 43.0 µg a.s./L, so no dose response relationship was apparent and therefore this observation was not used to establish the overall NOEC. Effects on pigmentation were seen at all concentrations but were not recorded at all observation points and not considered to represent an adverse effect of the test item. Based on the specific growth rate the EC_{20} was calculated to be 30.74 µg a.s./L. The overall NOEC for the study was concluded to be 19.2 µg a.s./L, based on the biological observations in the study and primarily the calculated EC_{20} for growth rate. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Overall conclusion

There are sufficient suitable studies to allow classification of the chronic/long-term hazard to fish from exposure to diflufenican and **overall the key fish endpoint to use for hazard classification purposes is considered to be the EC_{10} = 5.43 µg a.s./L, from Anon. 2007c**.

11.6.2 Chronic toxicity to aquatic invertebrates

Long-term studies were provided both for the original approval for diflufenican under Dir. 91/414/EEC, and for the renewal of the active under Reg. (EC) 1107/2009 (circa 2018). Those studies considered acceptable are summarised below.

Putt (2000). Diflufenican – The chronic toxicity to *Daphnia magna* under static-renewal conditions.

The chronic toxicity of diflufenican to *D. magna* (≤ 24 hours old) was assessed in a study performed to the OECD 211 guideline and in accordance with GLP. Exposure to the test item was for 21 days under static-renewal conditions, at nominal concentrations of 0 (control), 31, 63, 130, 250 and 500 µg a.s./L (a solvent control was also tested). Test solutions were renewed on days 3, 5, 7, 10, 12, 14, 17 and 19. Mean measured treatment concentrations were established at 24, 52, 96, 150 and 260 µg a.s./L, the mean measured treatment concentrations were used to establish the relevant endpoints for the study.

No statistically significant difference between the pooled control and treatment groups was reported in relation to parental survival. The number of young produced at 260 µg a.s./L was significantly different to the pooled controls. Mean total body length was statistically significantly different from the pooled controls at 150 and 260 µg a.s./L. Mean dry weight at test termination was statistically significantly different from the control at 96, 150 and 260 µg a.s./L. On the basis of the effect on dry weight the overall NOEC was set at 52 µg a.s./L. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Burke (2007b). Diflufenican technical: Prolonged toxicity to *Daphnia magna*.

The chronic toxicity of diflufenican to *D. magna* (≤ 24 hours old) was assessed in a study performed to the OECD 211 guideline and in accordance with GLP. Exposure to the test item was for 21 days under semi-static conditions, at nominal concentrations of 0 (control), 6.25, 12.5, 25, 50 and 100 µg a.s./L (a solvent control was also tested). Test solutions were renewed on days 2, 5, 7, 9, 12, 14, 16 and 19. The test item was not maintained within ± 20 % of the nominal concentration during the study; therefore mean measured treatment concentrations were established at 6.32, 1.4, 22.2, 43.1 and 78.1 µg a.s./L, the mean measured treatment concentrations were used to establish the relevant endpoints for the study. It is noted that analytical verification was not performed in all fresh / aged solutions; specifically the following were not measured days 2, 5, 9, and 19 in fresh solutions and days 5, 7, 14 and 21 in aged solutions. However, given that; 1) the renewal of the test solutions was every 3 days; 2) most recoveries were within ± 20 % of the nominal (see Table); and 3) that the DT_{50} for diflufenican is 31.7 days (as established in the evaluation of diflufenican under Reg. (EC) 1107/2009, circa 2018); it was considered that the test item would be expected to be maintained in the un-sampled renewal solutions broadly in line with the available measured results. Therefore the study's results were considered suitable for further consideration based on the mean measured treatment concentrations.

Table 22: Measured concentrations of diflufenican

Nominal concentration [µg a.s./L]	Geometric mean # [µg a.s./L]	Measured concentration [µg a.s./L] [% recovery of nominal concentration]							
		day 0 (fresh)	day 2 (old)	day 7 (fresh)	day 9 (old)	day 14* (fresh)	day 16 (old)	day 16 (fresh)	day 19 (old)
Control	-	nd	nd	nd	nd	nd	nd	nd	nd
Solvent control	-	nd	nd	nd	nd	nd	nd	nd	nd

Nominal concentration [µg a.s./L]	Geometric mean # [µg a.s./L]	Measured concentration [µg a.s./L] [% recovery of nominal concentration]							
		day 0 (fresh)	day 2 (old)	day 7 (fresh)	day 9 (old)	day 14* (fresh)	day 16 (old)	day 16 (fresh)	day 19 (old)
6.25	6.35	5.58 (89%)	5.10 (82%)	6.29 (101%)	5.48 (88%)	*5.01 (80%)*	6.18 (99%)	9.14 (146%)	7.26 (116%)
12.5	12.4	11.2 (90%)	12.1 (97%)	10.7 (86%)	11.1 (89%)	*7.69 (62%)*	12.6 (101%)	13.1 (105%)	16.5 (132%)
25	22.2	18.5 (74%)	15.0 (60%)	29.7 (119%)	20.1 (80%)	*13.1 (52%)*	22.4 (90%)	26.3 (105%)	26.9 (108%)
50	43.1	42.6 (85%)	39.5 (79%)	45.6 (91%)	37.3 (75%)	*12.8 (26%)*	37.7 (75%)	51.5 (103%)	49.8 (100%)
100	78.1	86.0 (86%)	45.6 (46%)	88.8 (89%)	67.8 (68%)	*50.4 (50%)*	64.5 (65%)	101 (101%)	115 (115%)

* Anomalous results obtained therefore data excluded from calculations
# Calculated excluding day 14 (fresh) analysis. (The geometric mean concentrations when including day 14 fresh samples are 6.14, 11.6, 20.8, 37.0 and 73.9).
nd = none detected
Fresh: freshly prepared media; Old: expired media

Ten percent parental mortality was reported in the solvent control and 12.4 µg a.s./L treatment group but this is within the test guideline's validity criteria, no parental mortality was reported in the other control or treatment groups. No statistically significant effects on body length or numbers of live neonates, at study termination, were reported for any treatment group. However, there were reported statistically significant differences in the numbers of dead neonates in treatment groups ≥ 43.1 µg a.s./L compared to the solvent control. The NOEC was therefore set at 22.2 µg a.s./L, due to the observations of dead neonates. The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

McElligot (1996b). Diflufenican: Toxicity to the sediment dwelling chironomid larvae (*Chironomus riparius*) under static conditions – 28 days.

The chronic toxicity of diflufenican to *C. riparius* was assessed in a study performed to a BBA (1995) guideline and in accordance with GLP. The study was conducted in a water sediment test system with the test item added to the overlying test water (200 g sediment with a depth of ~ 2 cm and 2.5 L test solution with a depth of ~ 20 cm, in 3 L beakers (10 – 13 cm diameter, 27.5 cm high)). Exposure to the test item was for 28 days under static conditions, at nominal concentrations of 0 (control), 6.25, 12.5, 25, 50 and 100 µg a.s./L (a solvent control was also tested). Three replicates each containing 25 (1st instar) larvae were prepared per treatment group. Treatment concentrations in the overlying water were not maintained within ± 20 % of the nominal during the test, the study's endpoints have therefore been established based on both initial measured and mean measured treatment concentrations in the water phase (calculated according to OECD 23).

No effects were observed in any treatment group (based on the measured parameters emergence ratio and development rate). Therefore the study's NOEC is equivalent to the maximum tested treatment concentration 100 µg a.s./L (nominal), equivalent to 91.2 µg a.s./L (measured initial) or 12.4 µg a.s./L (geometric mean measured). It is noted that under the OECD 219 (2004) guidance the initial measured treatment concentration would be used preferentially as the output of a study investigating chronic effects on *C. riparius* in a water sediment system dosed via the water. This study predates that guidance, though it does broadly follow the same methodology. However, the concentration of the test item in the sediment was not measured during the study so the behaviour of the test item in the test system, and accumulation in the sediment, cannot be established. Additionally, effects in sediment are not yet considered within the hazard classification scheme. Therefore, the mean measured endpoint is proposed for use in hazard classification; though it is noted that

this approach is conservative as it would not account for any toxicological effects due to exposure via the sediment (no effects were observed during the study). Regardless of the endpoint selected it is noted that this study would not result in the critical endpoint for hazard classification (algae are the most sensitive taxa). The study was considered suitable for use in risk assessment during renewal of diflufenican under Reg. (EC) 1107/2009 and is also considered suitable for use in hazard assessment according to Reg. (EC) 1272/2008.

Overall conclusion

There are sufficient suitable studies to allow classification of the chronic/long-term hazard to aquatic invertebrates from exposure to diflufenican and **overall the key invertebrate endpoint to use for hazard classification purposes is considered to be NOEC = 12.4 µg a.s./L from McElligot (1996b)**.

11.6.3 Chronic toxicity to algae or other aquatic plants

The available studies related to algae and aquatic plants have been summarised in Section 11.5.3.

Overall conclusion

There are sufficient suitable studies to allow classification of the chronic/long-term hazard to algae and other aquatic plants from exposure to diflufenican and **overall the key algal/plant endpoint to use for hazard classification purposes is considered to be the E_rC_{10} = 0.157 µg /L from Wilby (2007g)**.

11.6.4 Chronic toxicity to other aquatic organisms

No chronic toxicity studies on other organisms were considered valid for use in the hazard assessment.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data regarding technical diflufenican are available for fish, invertebrates, algae and other aquatic plants (i.e. there is appropriate data for all three trophic levels that need to be assessed for CLP classification). The lowest LC_{50}/EC_{50} value is the measured 72-hour E_rC_{50} of 0.0006 mg a.s./L for the alga *R. subcapitata* (Wilby, 2007g). This is > 0.0001 mg/L but ≤ 0.001 mg/L, therefore diflufenican should be classified as 'Aquatic Acute Category 1' with an acute M-factor of 1000.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Bioaccumulation

The measured Log K_{ow} for diflufenican is 4.2 which is greater than the CLP trigger of ≥ 4 indicating a potential for bioaccumulation. Two high quality studies are available to establish measured BCF estimates (Anon. 1998a; Anon. 2008). According to the guidance (ECHA, 2017) measured estimates should be used in preference when available to conclude on the bioaccumulation potential of a substance (BCF ≥ 500 indicates bioaccumulation potential). Therefore, these data have been used to conclude on the potential for bioaccumulation of diflufenican. The relevant whole fish 5 % lipid normalised BCF estimates are 1650 L kg^{-1} (Anon. 1998a) and 2583 L kg^{-1} (Anon. 2008) respectively. Given that there are fewer than four estimates a geometric mean value has not been established. Both BCF estimates are greater than 500 and as such it is concluded that diflufenican meets the CLP criterion and possesses the potential for bioaccumulation according to Reg. (EC) 1272/2008.

Degradation

Diflufenican is considered to be 'not rapidly degradable' according to the CLP criteria (see Section 11.2.1).

Chronic toxicity

Long-term aquatic toxicity data regarding technical diflufenican are available for fish, invertebrates, algae and other aquatic plants (i.e. there is appropriate data for all three trophic levels that need to be assessed for CLP classification). The lowest NOE_rC value is the measured 72-hour NOE_rC of 0.000095 mg a.s./L for *R. subcapitata* (derived from Wilby 2007g); the lowest measured 72-hour E_rC_{10} value is 0.000157 mg a.s./L for *R. subcapitata* (also derived from Wilby 2007g). Based on the 'Guidance on the Application of the CLP Criteria' (ECHA, 2017) the E_rC_{10} value has been used preferentially over the NOE_rC to determine the chronic hazard classification, therefore the relevant endpoint is 0.000157 mg a.s./L. This is > 0.0001 mg/L but ≤ 0.001 mg/L, and since diflufenican is considered to be 'not rapidly degradable' as well as potentially bioaccumulative, it should be classified as 'Aquatic Chronic Category 1' with a chronic M-factor of 100. Since the lowest algal E_rC_{10} and NOE_rC values straddle the boundary between chronic M-factors of 100 and 1000, the RAC may wish to consider this choice of endpoint.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

The current harmonised classification of diflufenican is 'Aquatic Chronic 3; H412'. The available studies included in this report indicate that a revised hazard classification is required to address the sensitivity of algae to diflufenican. Based on the information evaluated above; diflufenican exhibits the potential to bioaccumulate according to the CLP classification criterion (measured BCF ≥ 500); is considered to be 'not rapidly degradable'; and 3) is sufficiently toxic to warrant the highest CLP classifications for both acute and chronic hazards to the aquatic environment.

Classification with:

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

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Diflufenican has for environmental hazard a current Annex VI entry with a harmonised classification as Aquatic Chronic 3; H413.

The DS proposed to classify diflufenican as Aquatic Acute 1; H400 with an M-factor of 1 000 and as Aquatic Chronic 1; H410 with an M-factor of 100.

Rapid degradability

The dossier submitter proposed to consider diflufenican as <u>not</u> rapidly degradable for classification purposes. The basis for this proposal is that all the available information on the behaviour of diflufenican in water (hydrolysis, photolysis, aerobic mineralisation in surface water and natural water/sediment studies) indicate that diflufenican is stable to hydrolysis, undergoes very slow primary degradation in water or sediment and has

enhanced but slow degradation under illumination.

Three aqueous hydrolysis studies were submitted (Reeves and Savege, 1985; Reeves and Savege, 1986; Juozenaite, 2008). All three studies showed consistent behaviour for diflufenican to be stable to hydrolysis even at environmental unrealistic temperature of 50 °C.

In two ready biodegradability studies (Lebertz, 1989, OECD TG 301D; Desmares-Koopmans, 2008, OECD TG 301B). Diflufenican is not readily biodegradable with only 5.2 % and 9 to 21 % biodegradation after 28 days. Both studies were conducted appropriately and to GLP; they are thus considered to be reliable.

Two reliable aerobic mineralisation simulation studies in surface water, conducted to OECD TG 309 and GLP compliant, are available for diflufenican (Hein and Kasel, 2016; Ilieva, 2016b). Both studies showed consistent results for diflufenican with virtually no degradation.

Five valid water/sediment studies are available for diflufenican (Knoch, 1996; Crowe, 2003; Unsworth, 2006; Mamouni, 2003; Adam, 2008. All were conducted according to OECD TG 308 or other internationally recognised guidelines very similar to OECD TG 308. They indicate that significant partitioning from water into sediment occurs. The decline of diflufenican in the water layer was primarily due to partitioning to sediment. Primary degradation is slow in the entire water/sediment systems. The kinetics in the whole system were typically biphasic with DT_{50} values ranging from 86 up to 1 000 days and DT_{90} values of 341 up to 1 000 days.

Overall, the information on degradation show that diflufenican is not degraded under any environmentally realistic conditions. Consequently, diflufenican is considered to be 'not rapidly degradable' according to the CLP criteria.

Aquatic Bioaccumulation

The dossier submitter proposed to consider diflufenican as having a high bioaccumulation potential in the aquatic environment for classification purposes. The basis for this proposal is two studies with experimentally determined whole-fish, lipid-normalised BCF values of 1 650 L kg^{-1} and 2 583 L kg^{-1} and a measured Log K_{ow} of 4.2 at 20 °C.

Acute Aquatic Toxicity

The dossier submitter proposed to classify diflufenican as Aquatic Acute 1; H400 for the aquatic environment with an M-factor of 1 000. The basis for this proposal are acute studies that were provided both for the original approval for diflufenican under Dir. 91/414/EEC, and for the renewal of the active under Regulation (EC) 1107/2009. The dossier submitter selected only those studies of highest reliability/ quality. The studies have been evaluated, considered reliable and deemed suitable for hazard classification purposes. Only studies testing the technical substance, diflufenican, have been selected and studies testing any kind of formulation have not been considered.

For fish, there are sufficient suitable studies to allow classification of the acute hazard. The endpoint selected for use in hazard classification is LC_{50} > 0.0985 mg a.s./L from an OECD TG 203 acute toxicity test (96-hour) with common carp (*Cyprinus carpio*) under static conditions from Anon. (1998c). It is noted that fish are not the most acutely

sensitive taxa and therefore not critical for setting the hazard classification.

For aquatic invertebrates, there are sufficient suitable studies for classification purposes. The endpoint selected for use in hazard classification is EC_{50} > 0.240 mg a.s./L from an OECD TG 202 acute toxicity test (48 hours) with (*Daphnia magna*) under static conditions by Odin-Feurtet (1999f). It is noted that aquatic invertebrates are not the most acutely sensitive taxa and therefore not critical for setting the hazard classification.

For algae and other aquatic plants, there are sufficient suitable studies for classification purposes. The endpoint selected for use in hazard classification is the E_rC_{50} = 0.0006 mg a.s./L from an algal growth inhibition assay (OECD TG 201) by Wilby (2007g), with *Raphidocelis subcapitata* (formerly *P. subcapitata*, as referred to in the report, and *S. capricornutum*). Consequently, algae are the most acutely sensitive taxa and therefore are critical for setting the hazard classification.

However, the dossier submitter noted that *R. subcapitata* may not represent the most sensitive algal species. Within the dossier submitted to support diflufenican under Reg. (EC) 1107/2009 a study was available testing *Ankistrodesmus falcatus* that exhibited an E_rC_{50} of 0.000064 mg a.s./L, approximately 10 fold lower than that for *R. subcapitata*. The study was not considered valid due to failure to meet the validity criterion related to the mean coefficient of variation for section-by-section growth rates specified in the OECD TG 201. *A. falcatus* is a novel test species not normally used under OECD TG 201 and it was noted in the study report that there appeared to be a lag phase to the development of the cultures which may have explained the failure to meet the validity criterion. However, the study was not considered valid and so has not been used for hazard classification, but it is highlighted that this information suggests that other test species may exhibit greater sensitivity to diflufenican than *R. subcapitata*.

For other aquatic organisms there is one study on African clawed frog (*Xenopus laevis*) available. Since no specific guideline was available for this species the test protocol was based on the guidelines OECD TG 203 and US EPA 850.1075. The study was conducted according to GLP. The LC_{50} is > 0.0705 mg a.s./L and the NOEC is 0.0705 mg a.s./L indicating that it is not the most acutely sensitive species and therefore not critical for setting the hazard classification.

Chronic Aquatic Toxicity

The dossier submitter proposed to classify diflufenican as Aquatic Chronic 1; H410 for the aquatic environment with an M-factor of 100, based on studies meeting the criteria already described in the introductory paragraph for Acute Aquatic Toxicity.

For fish, there are sufficient suitable studies to allow classification for chronic/long-term hazard. The endpoint selected for use in hazard classification is the EC_{10} = 0.00543 mg a.s./L (length and mean measured concentrations) from a fish early life stage toxicity test (OECD TG 201) for *Fathead minnow* by Anon. (2007c). It is noted that fish are not the most long-term sensitive taxa and therefore not critical for setting the hazard classification.

For aquatic invertebrates, there are sufficient suitable studies to allow classification for chronic/long-term hazard. The endpoint selected for use in hazard classification is the NOEC = 0.0124 mg a.s./L (geometric mean measured) for sediment dwelling chironomid larvae (*Chironomus riparius*) by McElligot (1996b). It is noted that aquatic invertebrates

are not the most long-term sensitive taxa and therefore not critical for setting the hazard classification.

For algae and other aquatic plants, there are sufficient suitable studies to allow classification for chronic/long-term hazard. The endpoint selected for use in hazard classification is the E_rC_{10} = 0.000157 mg /L from an Algal growth inhibition assay (OECD TG 201) by Wilby (2007g), this is based on the most sensitive taxon to *Raphidocelis subcapitata* (formerly *P. subcapitata*, as referred to in the CLH report, and *S. capricornutum*). Consequently, algae are the most long-term sensitive taxa and therefore are critical for setting the hazard classification.

No chronic toxicity studies on other organisms were considered valid for use in the hazard assessment.

Comments received during public consultation

The public consultation obtained three comments from MSCAs and one comment from industry on the proposals for environmental classification. All three MSCAs agreeing with the proposed classification for diflufenican as Aquatic Acute 1; H400 with an M-factor of 1 000 and as Aquatic Chronic 1; H410 with an M-factor of 100.

RAC agrees that 20 °C or higher test temperature may not be environmental realistic temperatures for European surface water bodies and that DT_{50} values should always be normalised to 12 °C as it was agreed within RAC to be more environmentally realistic temperatures. However, in the case of diflufenican, RAC notes that this deficit clearly does not affect the conclusion on rapid degradability.

RAC notes the comment that the normalised BCF value for the Anon. (2008) study may be unreliable. RAC has not assessed this further because this would not impact the conclusion that diflufenican has a high potential to bioaccumulate.

Additional key elements

During the preparation of the first draft opinion, RAC became aware of additional information generated during the procedure for renewal of the approval of Diflufenican in accordance with Commission implementing regulation (EU) No 844/2012. Additional information was submitted in March 2018 referring mainly to supplementary supporting information on both environmental fate and toxicity. Due to several limitations, the studies have not been taken into account by RAC for the purpose of classification.

However, one new experimental study on *Ankistrodesmus falcatus* has been assessed by RAC as being relevant for the purpose of classification.

Eckenstein (2016): Diflufenican Tech: Toxicity to *Ankistrodesmus falcatus* in a 72-hour algal growth inhibition test supplemented with testing for recovery of growth (IES Study 20160072). The freshwater green algae *Ankistrodesmus falcatus* was exposed to Diflufenican for 72 hours under static exposure conditions. The test was carried out in accordance with OECD TG 201. The recovery of algal growth after the exposure period was recorded for two recovery periods of three days each resulting in six days recovery in total. The nominal test concentrations were 0.000022-0.001 mg/L, with a control and a solvent control group tested in parallel. Measured concentrations were maintained within

91 to 125 % (the latter at the lowest tested concentration only) and therefore, reported results were based on nominal test concentrations. All test validity criteria have been fulfilled.

The study recorded a 72-h EC_{50} value of 0.000071 (0.000067-0.000075) mg/L based on growth rate and a 72-h EC_{50} value of 0.000039 (0.000037-0.000040) mg/L based on yield.

It also reported a 72-h EC_{10} value of 0.000029 (0.000026-0.000033) mg/L based on growth rate and a 72-h EC_{10} value of 0.000025 (0.000023-0.000027) mg/L based on yield. The respective NOEC values were 0.000022 mg/L for both growth rate and yield.

Finally, complete recovery of growth of the algae was demonstrated for all test concentrations at the latest after 6 days in test water free of test item.

ECHA has launched a targeted public consultation, which has ended on 15 April 2019. This targeted public consultation obtained four comments from MSCAs. All MSCAs supported the reliability, validity and robustness of the new algae study for the purpose of classification.

One comment from industry questioning the appropriateness of the species selected. They argued that *Ankistrodesmus falcatus* is not a standard species for testing of algae toxicity under laboratory conditions, it has not been described as a test species in the current OECD TG 201 and no standardized and validated test conditions are available. However, from scanning the full REACH database for reliable experimental studies performed on *Ankistrodesmus falcatus*, it was found that this species has been mentioned as test organism 39 times in all IUCLID REACH registration dossiers, covering 24 unique dossier UUIDs (meaning that some dossiers have used it in multiple studies). The number of unique EC substances covered in these dossiers is 22. RAC notes that although *Ankistrodesmus falcatus* is not listed in annex 2 of OECD TG 201 (strain shown suitable for the test) many other species may be used in this test system. *Ankistrodesmus falcatus* has been used in OECD TG 201 before and RAC sees no need to dismiss this species.

IND criticised that the growth of *A. falcatus* in a standard growth inhibition test over 72 hours seems to be rather slow compared to standard algae test species and that high variability is observed in the reported growth rates during the main test including both recovery periods. RAC cannot confirm these arguments. The biomass increase is 38 fold and way above the validity criteria of ≥ 16. Neither growth rate nor variability gives reasons to consider this study as unreliable. The test has been conducted according to OECD TG 201. All validity criteria were met, exposure concentrations were maintained, and a clear dose-response was observed.

IND considered that the final result of this study is inconsistent compared to the corresponding range finder results and sees doubt on the scientific robustness of the study. The range finder (study report 20160075) resulted in 0.5 %, 27 % and 90 % growth inhibition at the test levels of 0.01, 0.1 and 1.0 µg/L, respectively. IND claimed, that based on these results the E_rC_{50} could be assumed to be around 0.2 µg/L. RAC notes that in general inconsistency compared to range finder results is not on its own an arguments to question the scientific robustness of a study and to dismiss a study result.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal of the dossier submitter to consider diflufenican as not rapidly degradable for classification purposes, based on three aqueous hydrolysis, two ready biodegradability, two aerobic mineralisation in surface water and five water/sediment studies that all slowed very slow biotic and abiotic degradation.

Aquatic Bioaccumulation

RAC agrees with the proposal of the dossier submitter to consider diflufenican as having high bioaccumulation potential in the aquatic environment for classification purposes, based on two studies with experimentally determined whole-fish, lipid-normalised BCF values of 1 650 L kg^{-1} and 2 583 L kg^{-1} and a measured Log K_{ow} of 4.2 at 20 °C.

Acute Aquatic Toxicity

RAC evaluates the new available OECD TG 201 study on *Ankistrodesmus falcatus* by Eckenstein (2016) as valid, robust and relevant for acute classification. RAC concludes to base the acute classification on the 72-h EC_{50} value of 0.000071 mg/L (growth rate) and that diflufenican warrants **classification as Aquatic Acute 1; H400 with an M-factor of 10 000.**

Chronic Aquatic Toxicity

RAC evaluates the new available OECD TG 201 study on *Ankistrodesmus falcatus* by Eckenstein (2016) as valid, robust and relevant for chronic classification. RAC concludes to base the chronic classification on the 72-h EC_{10} value of 0.000029 mg/L (growth rate), to consider diflufenican as not rapidly degradable and as having high potential for bioaccumulation. RAC concludes that **diflufenican warrants classification as Aquatic Chronic 1; H410 with an M-factor of 1 000**.

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

There are no data provided regarding the hazard of diflufenican to the ozone layer, the Ozone Depleting Potential (ODP) of diflufenican has not been measured. Howevrer, diflufenican is a solid, with a corresponding extremely low vapour pressure (4.25 x 10-6 Pa at 25 °C) . No boiling point could be determined below 360 °C. Hence, it is unlikely that diflufenican would be available in the stratosphere. In addition, diflufenican does not contain any other halogen functionality other than fluorine.

12.2 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 physcio-chemcial properties, diflufenican is not expected to be hazardous to stratospheric ozone.

12.2.1 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified, data lacking.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The dossier submitter proposed not to classify diflufenican as hazardous to the ozone layer. The basis for this proposal is that there are no data provided regarding the hazard of diflufenican to the ozone layer and the Ozone Depleting Potential (ODP) of diflufenican has not been measured. However, diflufenican is a solid, with a corresponding extremely low vapour pressure (4.25×10^{-6} Pa at 25 °C). No boiling point could be determined below 360 °C. Hence, it is unlikely that diflufenican would be available in the stratosphere.

In addition, diflufenican does not contain any other halogen functionality other than fluorine. 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. Although no specific data have been provided for this hazard, considering the chemical structure and other available information on the physico-chemical properties, diflufenican is not expected to be hazardous to stratospheric ozone.

Comments received during public consultation

No comments have been received on the DS's proposal to not classify diflufenican as

hazardous to the ozone layer.

Assessment and comparison with the classification criteria

RAC agrees with the proposal of the dossier submitter that diflufenican **does not warrant classification as hazardous to the ozone layer**.

13 ADDITIONAL LABELLING

No additional labelling is proposed.

14 REFERENCES

Author	Year	Reference
Physico-chemical references		
Muehlberger	2002a	Physical characteristics color, appearance and odor Diflufenican substance pure and substance technical Report No.: OE02/125, Not GLP, Unpublished
Bascou, J. P.	1998	Diflufenican: Physical characteristics Report No.: R&D/CRLD/An/9816584, GLP, Unpublished
Cicotti, M.	1992	Determination of the vapour pressure of diflufenican in accordance with EPA 63-9 and OECD 104/EEC A.4 guidelines Report No.: M-172759-01-2, GLP, Unpublished
Muehlberger	2002b	Partition coefficient 1-octanol / water (HPLC-method) Diflufenican Report No.: PA01/074, GLP, Unpublished
Francois, J. M.	1998	Determination of the explosion properties, flammability, ability for self heating of technical diflufenican Report No.: 98-225-SEC GLP, Unpublished
Bascou, J. P.	1999	MB38181: N-octanol / water partition coefficient Report No.: R006564,

		GLP, Unpublished
Muehlberger, B.	2003	AE 0542291 (MB043625) Determination of the Water Solubility (flask method) Report No.: PA03/021 GLP, Unpublished
Human health references		
Anon.1985	1985	Effect of *anon.* on reproductive function of multiple generations in the rat GLP, unpublished
Anon. 1987	1987	Addendum to *anon*. - Effect of *anon.* on reproductive function of multiple generations in the rat - Histopathology of the reproductive tract in F0 and F1A adult rats. GLP, unpublished
Anon. 1984a	1984a	Effect of *anon*. on pregnancy of the rat GLP, unpublished
Anon. 2002	2002	Teratogenicity study in wistar rats with diflufenican technical GLP, unpublished
Anon. 1984b	1984b	Effect of *anon.* on pregnancy of the rabbit GLP, unpublished
Anon. 1983	1983	Herbicides: *anon.* : Two-week toxicity study by the oral route in rats. Non-GLP, unpublished
Anon. 2001	2001	Diflufenican technical : 28 day dietary range finding study in wistar rats. GLP, unpublished
Anon. 1984c	1984	*Anon.*: 13 week toxicity study in rats by the dietary route GLP, unpublished
Anon. 1985a	1985	*Anon.*: Repeat 13 week dietary toxicity study in rats. GLP, unpublished
Anon. 1987a	1987a	*Anon.*: Toxicity study by dietary administration to F-344 rats for 13 weeks followed by 4 and 8 week reversibility periods. GLP, unpublished
Anon. 1987b	1987b	*Anon.:* Toxicity study by dietary administration to F-344 rats followed by 4 and 8 weeks reversibility periods First supplement to LSR Report *Anon.* - Electron microscopy report of selected hepatic tissues from the 13 week sacrifice. Non-GLP, unpublished
Anon. 2002a	2002	Diflufenican technical : Repeated dose 90 day oral toxicity study in wistar rats GLP, unpublished

Anon. 1993	1993	Diflufenican: Toxicity study by dietary administration to B6C3F1 mice for 13 weeks. GLP, unpublished
Anon. 1984c	1984	Herbicide – *Anon.* : 13-week oral toxicity in dogs Non-GLP, unpublished
Anon. 1987c	1987c	*Anon.:* 52-week toxicity study in oral administration to beagle dogs GLP, unpublished
Anon. 1987d	1987	*Anon.*: Combined oncogenicity and toxicity study in rats GLP, unpublished
Anon. 1986	1986	*Anon.*: Combined oncogenicity and toxicity study in mice Final report. GLP, unpublished
Fate and Behaviour References		
Reeves G.L., Savage E.A	1985	Diflufenican-14C: Hydrolysis in aqueous conditions at 22 degrees Celsius. Generated by: Rhone-Poulenc; May & Baker Ltd., GBR; Environmental Chemistry Document No: **R008078** GLP Yes, unpublished
Reeves G.L., Savage E.A	1986	Diflufenican-14C: Hydrolysis in aqueous conditions at 50 degrees and 70 degrees Celsius. Generated by: Rhone-Poulenc; May & Baker Ltd., England; Environmental Chemistry GLP, unpublished
Juozenaite A	2008	Diflufenican - Hydrolysis Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom ADAMA Agan Ltd., Report No.: AGM0327, GLP, unpublished
Simmonds M.B., Mills E.A.M.	2002a	(14C)-Diflufenican: Aqueous photolysis and quantum yield at pH7 Generated by: BCS S.A., FRA; Battelle-AgriFood Ltd., Essex, GBR; GLP, unpublished
Simmonds M.B., Mills E.A.M.	2003a	(14C)-Diflufenican: Aqueous photolysis and quantum yield at pH7 (amendment) Generated by: BCS S.A., FRA; Environmental Chemistry, Lyon Battelle-AgriFood Ltd., Essex, GBR; GLP, unpublished
Buntain I.G.	2003a	Diflufenican: Estimation of environmental photolytic half-life in water Model calculation according to Frank and Kloepffer Generated by: Battelle AgriFood Ltd., Essex, GBR; BCS S.A., FRA; Environmental Chmiestry, Lyon GLP, unpublished
Brands C.I.	2008	Photochemical degradation of diflufenican [pyridine ring-2-^{14}C] in water NOTOX B.V.

		Report No. 486561 GLP, unpublished
Derz, K.	2012	Phototransformation of diflufenican in water – direct photolysis Report No.: EUR-003/7-05 Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany GLP, unpublished
Unsworth R	2007	Report amendment 1 - Diflufenican - Photodegradation in water and determination of the quantum yield Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom ADAMA Agan Ltd., Report No.: AGM0277, Edition Number: M-554362-02-1 Date: 2007-07-27 ...Amended: 2009-01-08 GLP/GEP: yes, unpublished
Mills EAM, Simmonds MB	2002	(14C)-Diflufenican: Aqueous photolysis in natural water Battelle AgriFood Ltd., Ongar, Essex, United Kingdom Bayer CropScience, Report No.: C026218, GLP, unpublished
Lebertz, H	1989	Diflufenican: Biodegradability according to closed bottle test, (OECD guideline 301D for testing chemicals) Generated by: Rhone-Poulenc; Battelle Europe, Frankfurt; Rhone-Poulenc Agro GmbH, Kerpen, Germany; Document No: **R003371** GLP, unpublished
Desmares-Koopmans, M. J. E.	2008	Determination of 'ready' biodegradability: Carbon dioxide (CO2) evolution test (modified sturm test) of diflufenican technical NOTOX B.V. Report No. 486562 GLP, unpublished
Hein EM, Kasel D	2016	[trifluoromethylphenyl-UL-14C]diflufenican: Aerobic mineralization in surface water - Final report - Bayer CropScience, Report No.: EnSa-15-0906, GLP, unpublished
Ilieva, D	2016b	Aerobic mineralisation of [F2-phenyl-U-^{14}C]diflufenican in surface water Report No.: S15-06023 Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH, Niefern-Öschelbronn, Germany GLP, unpublished
Knoch E	1996	Degradation and metabolism of diflufenican in water / sediment systems Generated by: Rhone-Poulenc; Rhone-Poulenc Secteur Agro; Analytical Chemistry ORS Institut Fresenius Chem.und Biolog. Lab. GmbH; Document No: **R000436** GLP, unpublished
Crowe A.	2003a	Diflufenican: Degradability and fate in the water/sediment system Generated by: Huntingdon Life Sciences Ltd., Suffolk, GBR; Eye

		Research Centre Huntingdon Life Sciences Ltd., Suffolk, GBR; Eye Research Centre BCS; Document No: **C031677** GLP, unpublished
Unsworth R	2006	Diflufenican - Degradability and fate in the water/sediment system Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom ADAMA Agan Ltd., Report No.: AGM0228, Edition Number: M-554363-01-1 Date: 2006-09-06 GLP, unpublished
Mamouni A	2003	14C-Diflufenican: Route and rate of degration in aerobic aquatic systems RCC Ltd., Itingen, Switzerland TF – Bayer – Adama- Cheminova Report No.: 844263 GLP, unpublished
Adam D.	2008	^{14}C-Diflufenican Route and Rate of Degradation in Aerobic Aquatic Sediment Systems Harlan Laboratories Ltd. Report No. B63674 GLP, unpublished
Giraud J, P, Plewa, A	1984	Diflufenican - (MB 38544) - Soil sorption study Rhone-Poulenc Agro, Lyon, France Bayer CropScience, Report No.: R006369, GLP, unpublished
Simmonds, M.; Brett, R.	2006	[14C]-Diflufenican: Adsorption to and desorption from six soils Battelle UK Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/05/083, Document Number: **M-278751-01-1** GLP, unpublished
Knight L	2008a	Diflufenican - Adsorption / Desorption on five soils Huntingdon Life Sciences Ltd., Huntingdon, United Kingdom ADAMA Agan Ltd., Report No.: AGM0314, GLP, unpublished
Traub, M.	2012a	Determination of the adsorption/desorption behaviour of diflufenican in six soils Report No.: S09-02949 Eurofins Agroscience Services EcoChem GmbH, Niefern-Öschelbronn, Germany GLP, un published
Ecotoxicity References		
Anon. 2013	2013	Acute toxicity of diflufenican technical to the African clawed frog (*Xenopus laevis*) under static conditions. Unpublished.
Anon. 1997	1997	Fish, juvenile growth test 28 days under flow-through conditions

		Unpublished.
Anon. 1998a	1998a	(^{14}C)-Diflufenican: Bioaccumulation and metabolism in rainbow trout. Unpublished.
Anon. 1998b	1998b	Diflufenican: Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) under static conditions. Unpublished.
Anon. 1998c	1998c	Diflufenican: Acute toxicity (96-hour) to common carp (*Cyprinus carpio*) under static conditions. Unpublished.
Anon. 1998d	1998a	Diflufenican - Early life-stage toxicity test with fathead minnow (*Pimephales promelas*). Unpublished.
Anon. 2007a	2007a	Diflufenican technical: Acute toxicity to fish rainbow trout. Unpublished.
Anon, 2007b	2007b	Diflufenican technical: Acute toxicity to fish Unpublished.
Anon. 2007c	2007a	Diflufenican technical: Fish early life stage toxicity test for fathead minnow. Unpublished.
Anon. 2008	2008	Diflufenican: Bioconcentration in rainbow trout. Unpublished.
Anon. 2007d	2007c	*Anon.*- Acute toxicity to fish – rainbow. Unpublished.
Anon 1996	1996	*Anon.* - Acute toxicity (96-hours) to rainbow trout (*Oncorhynchus mykiss*) under static conditions. Unpublished.
Anon. 2007e	2000d	*Anon.*: - Acute toxicity to fish. Unpublished.
Anon 2015	2015	*Anon.* - Acute toxicity to fish (*Oncorhynchus mykiss*) under static conditions. Unpublished.
Burke	2007b	Diflufenican technical: Prolonged toxicity to *Daphnia Magna*. Report number AGM0298/073902. Unpublished.
Douglas & Handley	1987c	The acute toxicity of diflufenican soil metabolite No.2, AE 0542291 (MB43625) to *Daphnia magna*. Report number M&B 268(b)/87724. Unpublished.
ECHA	2017	Guidance on the application of the CLP criteria: Guidance to regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. (Version 5.0).

Hermes & Emmet	2016	Diflufenican technical: Toxicity to the Aquatic Plant *Myriophyllum spicatum* in a Static Growth Inhibition Test with a Prior Rooting Phase. Report number 109761215. Unpublished.
Hoberg	1997a	Diflufenican technical - Toxicity to the freshwater diatom, *Navicula pelliculosa*. Springborn Laboratories Inc. Report number 97-10-7131. Unpublished.
Hoberg	1998f	Diflufenican - Toxicity to the freshwater blue-green alga, *Microcystis aeruginosa*. Springborn Laboratories Inc. Report number 98-4-7308. Unpublished.
Hoberg	1998h	Diflufenican - Toxicity to the duckweed, *Lemna gibba*. Springborn Laboratories Inc. Report number 98-4-7296. Unpublished.
Juckeland	2015a	Effects of diflufenican technical on *Chlamydomonas rheinhardtii* in an algal growth inhibition test with a recovery phase. Report number 151048016W. Unpublished
Juckeland	2015b	Effects of diflufenican technical on *Chlorella vulgaris* in an algal growth inhibition test with a recovery phase. Report number 151048083W. Unpublished.
Juckeland	2015c	Effects of diflufenican technical on *Desmodesmus communis* in an algal growth inhibition test with a recovery phase. Report number 151048015W (including Report amendment No. 1). Unpublished.
Juckeland	2015d	Effects of diflufenican technical on *Myriophyllum spicatum* in a water-sediment system. Report number 151048082W. Unpublished.
Kuhl	2015c	*Lemna gibba* G3 - Growth inhibition test with AE 0542291 (BCS-AI56853) under static conditions. Report number EBDCN098. Unpublished.

Kuhl	2015d	*Lemna gibba* G3 - Growth inhibition test with AE B107137 (BCS-AB27392) under static conditions. Report number EBDCN099. Unpublished.
Kuhl	2016a	Toxicity of diflufenican (AE F088657) to the aquatic plant *Myriophyllum spicatum* in a static growth inhibition test - Final report. Report number EBDCN163. Unpublished.
McElligot	1996b	Diflufenican: Toxicity to the sediment dwelling chironomid larvae (*Chironomus riparius*) under static conditions – 28 days. Rhône-Poulenc Agro. Report number SA 96086. Unpublished.
Mead & Mullee	2001a	AE 0542291 (MB 43625): Algal inhibition test. Report number 1392/017. Unpublished.
Odin-Feurtet	1998a	Diflufenican: Freshwater algal growth inhibition study in a sediment water system (*Scenedesmus subspicatus*). Rhône-Poulenc Agro. Report number SA 98022. Unpublished
Odin-Feurtet	1998c	Diflufenican: Freshwater algal growth inhibition study in a sediment water system (*Scenedesmus subspicatus*). Rhône-Poulenc Agro. Report number SA 98274. Unpublished.
Odin-Feurtet	1998f	Diflufenican: Acute toxicity (48 hours) to daphnids (*Daphnia magna*) under static conditions. Report number SA 98669. Unpublished.
Pavic	2008	Acute Toxicity of 2-[3-(trifluoro methyl) Phenoxy]nicotinamide to *Daphnia magna* in a Static 48-hour Immobilization Test. Report number 37862220. Unpublished.
Pavic & Wydra	2008	Acute Toxicity of 2-[3-(trifluoro methyl) Phenoxy]nicotinic acid to *Daphnia magna* in a Static 48-hour Immobilization Test. Report number 37873220. Unpublished.

Pupp & Wydra	2008b	Toxicity of 2-[3-(trifluoro methyl)phenoxy]nicotinic acid to *Desmodesmus subspicatus* in an algal growth inhibition test. Report number 37871210. Unpublished.
Pupp & Wydra	2008c	Toxicity of 2-[3-(trifluoro methyl)phenoxy]nicotinic acid to *Anabaena flos-aquae* in an algal growth inhibition test. Report number 37872210. Unpublished.
Pupp & Wydra	2008d	Toxicity of 2-[3-(trifluoro methyl)phenoxy]nicotinamide to *Desmodesmus subspicatus* in an algal growth inhibition test. Report number 37861210. Unpublished.
Pupp & Wydra	2008e	Toxicity of 2-[3-(trifluoro methyl)phenoxy]nicotinamide to *Anabaena flos-aquae* in an algal growth inhibition test. Report number 37867210. Unpublished.
Putt	2000	Diflufenican – The chronic toxicity to *Daphnia magna* under static-renewal conditions. Springborn Laboratories Inc. Study number 10566.6566. Unpublished.
Suteau	1996h	AE B107137 (MB38181) Acute toxicity (48 hours) to daphnids (*Daphnia magna*) under static conditions. Rhône-Poulenc Agro. Report number SA 96145. Unpublished.
v. Seydlitz-Kurzbach	2010a	Testing of effects of AE 0542291 on the single cell green alga *Pseudokirchneriella subcapitata* in a static 3 days test. Report number S09-03044. Unpublished.
Wilby	2007e	Diflufenican technical: Acute toxicity to *Daphnia Magna*. Report number AGM0289/073699. Unpublished.
Wilby	2007f	TFMP-NA (Acid) - Acute toxicity to *Daphnia magna*. Report number AGM0297/073756. Unpublished.
Wilby	2007g	Diflufenican technical: Algal growth inhibition assay. Report number AGM0290/073714. Unpublished.

Wilby	2007h	Diflufenican technical: Algal growth inhibition assay *Anabaena Spp*. Report number AGM0291/073713. Unpublished.
Wilby	2007i	Diflufenican technical: Algal growth inhibition assay *Navicula Pelliculosa*. Report number AGM0292/073700. Unpublished.
Wilby	2007k	Diflufenican technical: Higher plant (*Lemna Minor*) growth inhibition test. Report number AGM0293/073753. Unpublished.
Wilby	2007j	TFMP-NA (Acid) - Algal growth inhibition assay. Report number AGM0290/073757. Unpublished.
Wilby	2008a	TFMP-NA (amide): Algal growth inhibition assay. Report number AGM0351. Unpublished.

15 ANNEXES

15.1 Annex I

The available acute toxicity data available for the degradants of diflufenican (AE B107137 and AE 0542291) considered relevant under Reg. (EC) 1107/2009 are summarised below in

Table . This information is included for information only, none of the degradants exhibit equivalent toxicity to diflufenican or would require hazard classification in isolation. And so are not considered to impact the hazard classification of diflufenican.

Table 23: Summary of relevant information on acute aquatic toxicity

Species	Test material	Results[1]	Reference	Submitted for original approval (O) or renewal (N)
Fish				
O. mykiss	AE B107137	LC_{50} > 17300 µg /L (m.m.) [2]	Anon.1996	O
		LC_{50} > 90800 µg /L (m.m.)	Anon. 2007d	N
P. promelas		LC_{50} > 100000 µg /L (m.m.)	Anon. 2007f	N
O. mykiss	AE 0542291	LC_{50} > 8420 µg /L (m.m.)	Anon. 2015	N
Aquatic invertebrates				
D. magna	AE B107137	EC_{50} > 20400 µg /L (m.m.)	Suteau 1996h	O
		EC_{50} > 20000 µg /L (m.m.)	Pavic & Wydra 2008	N [3]
		EC_{50} > 85500 µg /L (m.m.)	Wilby 2007f	N
D. magna	AE 0542291	EC_{50} > 10000 µg /L (nom.) [4]	Douglas & Handley 1987c	O
		EC_{50} = 42100 µg /L (nom.)	Pavic 2008	N [3]
Algae				
Desmodesmus subspicatus (Formerly S. subspicatus)	AE B107137	E_rC_{50} > 20000 µg /L (nom.)	Pupp & Wydra 2008b	N
Anabaena flos-aquae		E_rC_{50} > 20000 µg /L (nom.)	Pupp & Wydra 2008c	N [3]
R. subcapitata (Formerly P. subcapitata and S. capricornatum)		E_rC_{50} > 87000 µg /L (ini.)	Wilby 2007j	N
D. subspicatus (Formerly S. subspicatus)	AE 0542291	E_rC_{50} = 66000 µg /L (nom.)	Mead & Mullee 2001a	O
		E_rC_{50} = 60160 µg /L (m.m.)	Pupp & Wydra 2008d	N [3]
R. subcapitata (Formerly P. subcapitata and S. capricornatum)		E_rC_{50} = 3376 µg /L (nom.)	v. Seydlitz-Kurzbach 2010a	N
		E_rC_{50} = 70800 µg /L (m.m.)	Wilby 2008a	N
Anabaena flos-aquae		E_rC_{50} > 64700 µg /L (m.m.)	Pupp & Wydra 2008e	N [3]
Other aquatic plants				
L. gibba	AE B107137	E_rC_{50} > 100000 µg /L (nom.)	Kuhl 2015c	N
L. gibba	AE 0542291	E_rC_{50} > 100000 µg /L (nom.)	Kuhl 2015d	N

Note: Data owners have been identified in square brackets for all new studies; new in the context of the most recent renewal of diflufenican under Reg. (EC) 1107/2009, circa 2018.

[1] m.m. = measured concentration; nom. = nominal concentration; ini. = initial measured

[2] Some treatment concentrations were above the limit of solubility, for at least part of the test duration.

[3] Full study evaluations were not conducted within the scope of Reg. (EC) 1107/2009, circa 2018, as the study was submitted as a data matching study. A brief check of the studies validity and endpoints was conducted but the study has not been fully evaluated. The quoted endpoints should not be used in hazard assessment without further consideration of the studies suitability.

[4] During the previous evaluation of the active substance, under Dir. 91/414/EEC, the results of this study were considered of questionable reliability due to the lack of analytical verification of the test item concentrations.

15.2 Annex II - confidential references (separate document)