Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl) amino}furan-2(5*H*)-one; flupyradifurone

EC Number: -
CAS Number: 951659-40-8

CLH-O-0000001412-86-228/F

Adopted

14 September 2018

14 September 2018

CLH-O-0000001412-86-228/F

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

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

Chemical name: **4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5*H*)-one; flupyradifurone**

EC Number: **-**

CAS Number: **951659-40-8**

The proposal was submitted by **the Netherlands** and received by RAC on **3 August 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **13 September 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **30 October 2017.**

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Sonja Kapelari**

Co-Rapporteur, appointed by RAC: **Pietro Paris**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one; flupyradifurone	-	951659-40-8	Repr. 2 Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361 H302 H373 (muscle) H400 H410	GHS07 GHS08 GHS09 Wng	H361 H302 H373 (muscle) H410		M=10 M=10	
RAC opinion	TBD	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one; flupyradifurone	-	951659-40-8	Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H373 (muscle) H400 H410	GHS08 GHS09 Wng	H302 H373 (muscle) H410		oral: ATE = 500 mg/kg bw M=10 M=10	
Resulting Annex VI entry if agreed by COM	TBD	4-{[(6-chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino}furan-2(5H)-one; flupyradifurone	-	951659-40-8	Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H373 (muscle) H400 H410	GHS08 GHS09 Wng	H302 H373 (muscle) H410		oral: ATE = 500 mg/kg bw M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Flupyradifurone is approved in the EU as an insecticide for plant protection products. Flupyradifurone interacts with insect nicotinic acetylcholine receptors, a target also known for neonicotinoid insecticides. EFSA (2015) informally proposed flupyradifurone to be classified as Acute Tox. 4; H301 and as Aquatic Acute 1 and Aquatic Chronic 1 (with no M-factors) in accordance with the provisions of Regulation (EC) No 1272/2008 (CLP).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The purified substance is a white powder at room temperature. The odour is weak, not characteristic. The melting point is 69.0 °C, the boiling point is 270 °C. No decomposition was observed prior to boiling. The vapour pressure of purified flupyradifurone is 9.1×10^{-7} Pa at 20 °C, which, combined with a low and pH dependent water solubility of 3.2 g/L at pH 7 in distilled water, results in a Henry´s law constant of 8.2×10^{-8} $Pa.m^3.mol^{-1}$.

Flupyradifurone has no bioaccumulative potential in lipophilic matrices (log K_{ow}: 1.2 at pH 7, 25 °C) and it is hydrolytically stable in aqueous solutions in the pH-range 1 < pH < 12 at ambient temperature. The substance is susceptible to reactions with hydroxyl radicals and to a lower extent with ozone in the troposphere.

The surface tension of technical flupyradifurone with a purity of 97.6 % is 69.1 mN/m at 20 °C. The material is not highly flammable, does not self-ignite and does not have oxidising or explosive properties. The chemical structure of the substance does not indicate any potential for self-reactivity.

In summary, no classification for physical hazard properties is proposed by the Dossier Submitter (DS).

Comments received during public consultation

One Member State Competent Authority (MSCA) commented on the classification for explosive properties. The appropriateness of the test battery for classification was contested, namely that the EU A.14 method for explosive properties does not entirely correspond to the CLP requirements. In order to validate the proposed classification, at least a "BAM Trauzl Test" should have been performed according to the specifications of test methods under the United Nation scheme.

Assessment and comparison with the classification criteria

The CLP Regulation (Section 2.1) and the CLP Guidance state that the classification for explosive properties is almost entirely adopted based on Part I of the UN Recommendations on the Transport of Dangerous Goods (UN RTDG; Manual of Tests and Criteria), which are appropriate for transport and also storage of packaged explosives. The classification of substances, mixtures and articles in the class of explosives and further allocation to a division is a very complex procedure.

Flupyradifurone was investigated under a test battery which cannot be directly related to the CLP regulatory text. However, the results proved negative in three relevant key areas: behaviour to heat, shock and friction.

In conclusion, RAC considers that there are sufficient data to conclude that flupyradifurone should not be classified for Explosive properties under the CLP Regulation although the exothermic decomposition energy of the substance is 895 J/g between 270 °C and 355 °C.

Consequently, the DS's proposal of **no classification for physical hazards** is supported.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS presented three studies performed with flupyradifurone (purity 96.2 %) in accordance with OECD Test Guidelines (TG) and GLP (good laboratory practice) for acute toxicity, one study for each route of exposure. Based on the outcome of these studies, the DS proposed to classify flupyradifurone as Acute Tox. 4 by the oral route (H302). The DS did not propose an Acute Toxicity Estimate (ATE, oral) for the purpose of classifying substances or mixtures containing flupyradifurone.

Comments received during public consultation

Three MSCAs agreed with the proposed classification. However, one of them pointed out that classification as Acute Tox. 4; H302 should be based on mortality at 2 000 mg/kg bw/day but not at 300 mg/kg bw/day.

Assessment and comparison with the classification criteria

Acute toxicity: oral

In the acute oral toxicity study, similar to OECD TG 423, two groups of three fasted female Wistar rats were given 300 mg/kg bw of flupyradifurone, two groups received a single dose of 2 000 mg/kg bw. In all rats given 300 mg/kg bw, breathing sounds from 50 minutes to 7 hours after exposure were noted. In the high-dose group, decreased motility, tremor, piloerection, laboured breathing and clonic cramps from 20 minutes to 5 days after dosing were observed. In the four animals that died after dosing with 2 000 mg/kg bw (one after 2 hours, the other three after 3 hours of exposure) gross pathological findings included black or black spotted liver and haemorrhagic lung. The dissection of the surviving animals did not show any particular findings.

Classification in Category 4 via the oral route, is required where the LD_{50} is > 300 and ≤ 2 000 mg/kg bw. Based on the rat acute oral toxicity study the LD_{50} for flupyradifurone is likely to be > 300 but lower than 2000 mg/kg bw. RAC agrees with the DS that flupyradifurone should be classified as Acute Tox. 4; H302 for the oral route. In addition, to facilitate consistent classification of mixtures containing flupyradifurone, a harmonised ATE value should also be proposed. According to the CLP regulation, the ATE value for a substance should be derived using the LD_{50} where available, which is not the case here. Therefore, the ATE is derived using the appropriate conversion value from Table 3.1.2 of CLP Regulation that relates to a classification category. The converted acute toxicity point estimate for a substance classified as Acute Tox. 4; H302 is 500 mg/kg bw. In conclusion, RAC is of the opinion that the converted ATE for

flupyradifurone should be used, and proposes to assign an ATE of 500 mg/kg bw for acute oral toxicity.

Acute toxicity: inhalation

The acute inhalation study has been carried out according to OECD TG 403 (1981), in which rats have been exposed during a period of 4 h (nose only). The test article was aerosolised as a 50 % (w/w) solution in PEG 400 (Lutrol). Clinical signs of toxicity after exposure to 4.7 mg/L (analytical concentration) flupyradifurone included increased and irregular breathing, laboured breathing patterns, stridor, piloerection, anxiety, tremor, exophthalmia, increased or reduced motility, high-legged gait, abdominal position with uncoordinated movements and disappeared in all animals 48 hours after exposure. Neither treatment related effects on body weight nor macroscopic pathologic abnormalities were observed.

Classification for acute toxicity via the inhalation route is required where the LC_{50} value is ≤ 5 mg/L (dusts and mists). The rat 4 h-LC_{50} for flupyradifurone is > 4.7 mg/L. RAC agrees with the DS that **no classification is warranted for acute inhalation toxicity**.

Acute toxicity: dermal

In the acute dermal toxicity study in rats, carried out according to OECD TG 402, a dose of 2 000 mg/kg bw moistened test material (flupyradifurone) was applied to 30 cm^2 of the rat skin under a semi-occlusive dressing. No signs of toxicity/treatment related findings were observed.

Classification for acute toxicity via the dermal route is required where the LD_{50} is ≤ 2 000 mg/kg bw. The LD_{50} was > 2 000 mg/kg bw. RAC agrees with the DS that **no classification is warranted for acute dermal toxicity**.

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

Summary of the Dossier Submitter's proposal

The DS did not propose any classification for STOT SE 1 or 2 as no toxicity to a specific organ were observed, neither in the acute toxicity studies in rats nor in a short-term oral neurotoxicity rat study. In each of these GLP-compliant studies conforming to OECD TG flupyradifurone (purity 96.2 %) was administered. In addition, the DS did not propose to classify flupyradifurone as STOT SE 3 for narcotic effects or respiratory tract irritation considering that no such effects were observed.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

In the acute oral and in the acute inhalation toxicity study in rats, the clinical signs were indicative of non-specific, general acute toxicity. Additionally, no acute organ toxicity was observed in a short-term oral neurotoxicity study, giving a LOAEL of 50 mg/kg bw and a NOAEL of 35 mg/kg bw based on piloerection and dilated pupils (females only).

All effects (e.g. rapid respiration, gait incoordination and flattened body posture at the time-of-peak effect after the dose of 200 mg/kg bw) were reversible. There were no macroscopic or microscopic treatment related observations in either sex at any dose level.

Overall, no specific target-organ toxicity was identified at doses equal to the top of the guidance value range listed in the CLP Regulation (Annex I: 3.8.2.1.9.3, Table 10.3.8.2). Accordingly, flupyradifurone does not meet the criteria for classification for STOT SE Categories 1 or 2 under the CLP Regulation.

STOT SE 3 is assigned for respiratory tract irritation (RTI) and/or narcotic effects. Flupyradifurone administrated by inhalation at a concentration of 4.7 mg/L for a period of 4 h induced general clinical signs of toxicity for a limited period of time (48 h) with no specific narcotic effects. Some findings are only suggestive of possible respiratory irritation (laboured and irregular breathing). Since flupyradifurone is not an irritant to the skin or the eyes, RAC agrees with the DS that there is no evidence to justify a classification for STOT SE 3. Overall, RAC agrees with the DS´s proposal that **no classification for STOT SE** is warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

According to the DS, flupyradifurone does not meet the criteria for classification as a skin irritant.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

In a standard GLP-compliant study similar to OECD TG 404 in rabbits using 0.5 g of pulverized flupyradifurone (purity 96.2 %) moistened with water, no irritative effects were seen at any time during the study.

RAC agrees with the DS that **no classification for skin irritation** is warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for eye damage.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

The eye damage/irritation potential of flupyradifurone was assessed in a GLP-compliant study, similar to OECD TG 405 (2002) with 0.1 g pulverized flupyradifurone (purity 96.2 %).

In all three animals conjunctival redness was observed at 1 and 24 h after dosing whereas chemosis was found only in one animal. The effects, however, were fully reversed after 48 h. The mean scores (24 to 72 h) were 0.7, 0.3 and 0.3, respectively, for conjunctival redness and 0.3, 0 and 0, respectively, for conjunctival chemosis, i.e. below the classification criteria.

RAC agrees with the DS that there is **no evidence to justify a classification for eye irritation**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for respiratory sensitisation in the absence of data on the potential of flupyradifurone to cause respiratory sensitisation.

Comments received during public consultation

No comments were provided.

Assessment and comparison with the classification criteria

A respiratory sensitiser is described as a substance that will lead to hypersensitivity of the airways. In the absence of any data, flupyradifurone **does not require classification for respiratory sensitisation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

According to the DS, flupyradifurone does not meet the criteria for classification as a skin sensitiser based on a GLP-compliant but non-guideline Murine Local Lymph Node Assay (LLNA) in mice.

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

The DS summarised in the CLH report a GLP-compliant skin sensitisation study similar to the LLNA based on lymph node cell counts (LNCC).

In that study, six female NMRI mice per group received 25 µL flupyradifurone (purity 96.2 %) at 0 %, 2 %, 10 % and 50 % epicutaneously onto the dorsal part of the ears. The treatment was repeated three times. One day after the last application, the animals were sacrificed. There were no signs of skin irritation. A positive control was not included.

The performed study is a variant of the LLNA (LLNA/IMDS (Integrated Model for the Differentiation of Skin reactions) which is based on LNCC, including the measurement of ear swelling after treatment): After weighing the removed auricular lymph nodes and crushing them

through a sieve, the cell counts were determined. Additionally, an 8 mm diameter section of ear was punched out and weighed.

In the LNCC assay, the stimulus index is calculated by dividing the mean node and ear weights, change in ear thickness (indicating the extent of ear swelling after treatment) and nodal cell counts by the mean values of these parameters in the vehicle control. There was no treatment related increase in any of the parameters which were evaluated up to and including a concentration of 50 % of the tested substance using an EC 1.4 value as the cut-off point between sensitisers and non-sensitisers.

Although the sensitising potential of the positive control (alpha hexyl cinnamic aldehyde) was demonstrated for the test system on a separate occasion in November 2008 (3 %, 10 %, 30 % - but not 50 % - in acetone/olive oil 4:1) and although Kolle *et al*. (2012) published an acceptable predictive value, RAC considers that the following uncertainties remain:

- publications on the LNCC indicate limitations and low predictability of the LNCC compared to the LLNA and other non-radioactive methods,
- the study does not include a positive control,
- historical control data from the performing laboratory were not provided,
- there is no information why the study was not performed with female mice of the usual CBA/Ca or CBA/J strains,
- there are no validated OECD TG for the LNCC study,
- there is no additional supporting information (e.g. human data, *in vitro*/*in chemico*/*in silico* tests).

However, after the public consultation, an OECD- and CLP-compliant LLNA in CBA/J mice (conducted in 2012 to satisfy non-EU requirements) was submitted by industry (Anon., 2012). After the applicability and biocompatibility test with concentrations of 25 % and 50 % (w/v) in DMF, the main assay was performed with 25 µL flupyradifurone (purity 96.2 %) at 10 %, 25 %, 50 % in dimethylformamide (DMF) applied epicutaneously onto the dorsal part of the ears on Days 1, 2 and 3. The study included a negative control group with DMF and a positive control group with 25 % alpha-hexyl cinnamaldehyde (HCA) in DMF. On Day 6, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine and the values obtained were used to calculate the stimulation indices (SI).

There were no signs of irritation seen at any dose level. The SI values at concentrations of 0 %, 10 %, 25 % and 50 % (w/v) were 1.0, 2.3, 2.5 and 1.6 whereas the positive control showed an SI value of 13.5.

Therefore, based on the LLNA study that was not included in the original CLH proposal from the DS, RAC is of the opinion that flupyradifurone does not warrant a classification for skin sensitisation.

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

Summary of the Dossier Submitter's proposal

The DS evaluated eight studies conducted by the oral route: four subacute (28-day) range-finding (non-GLP, non-guideline) studies in rats, mice and dogs performed in GLP-certified laboratories, three subchronic (90-day) in rats, mice and dogs (GLP- and OECD-compliant) and a 1-year chronic repeated toxicity study in dogs. In addition, the DS reported one subacute (28-day) dermal rat study, GLP-compliant and partly similar to OECD TG 410.

In the 90-day dog study, haemoglobin was not reduced up to 20 % in the high-dose group after dose reduction from 107 to 100 mg/kg bw/day. The DS therefore considered that this effect does not warrant classification for STOT RE 2. Also the increased liver enzymes which were found in the mid- and high-dose groups of this study were not considered to indicate specific liver toxicity, as there were no associated histopathological changes at the mid-dose group. This is supported by the fact that no effects on clinical chemistry were reported in the 1-year dog study at 28 mg/kg bw/day as this dose level is only slightly lower than the 90-day mid-dose level (33/41 mg/kg bw/day). The DS also highlighted that a decrease in Hb and haematocrit (Hct) values was also observed in female rats during a carcinogenicity study at 120 mg/kg bw/day. The decrease in Hb was at most 6.3 % at 6 months, which was significant, but below the guideline limits for classification. In the same study, slightly lower total bilirubin concentrations in both sexes and slightly higher mean total cholesterol in females were observed at 120 mg/kg bw/day. The DS reported that the most relevant toxicological effects were reduced body weight, reduced body weight gain, clinical chemistry abnormalities, statistically significantly increased kidney weight and focal myofiber atrophy/degeneration from the mid-dose level (33 mg/kg bw/day). The muscular effects were also observed in the 1-year dog study from a dose level of 28.1 mg/kg bw/day for males and 28.2 mg/kg bw/day for females. They are described as degeneration of the skeletal muscle (gastrocnemius) and of the biceps femoris (atrophy, necrosis and/or presence of inflammatory cells).

In the 90-day rat study, significant effects (clinical chemical findings, increased relative thyroid and liver weights) were observed at 30.2 mg/kg bw/day. However, these effects were not severe enough but at the highest dose (171 mg/kg bw/day) classification for STOT RE 2 would be warranted. An extrapolation to estimate these effects at 100 mg/kg bw/day is no possible. No target organ was proposed from the findings in rats.

Effects observed in mice studies do not warrant classification following repeated dose toxicity to flupyradifurone according to the DS. Information on repeated exposure in humans is not available.

Overall, the DS reported that flupyradifurone induced consistent, severe effects in dogs in repeated dose studies, below or around the guideline values for STOT RE 2. These effects included weight loss, minimal myofiber atrophy/degeneration, changes in haematology (> 20 % Hb reduction) and clinical chemistry parameters. The DS concluded that flupyradifurone warrants classification as STOT RE 2; H373 with "muscle" as a target organ.

Comments received during public consultation

Five MSCAs supported the proposal to classify flupyradifurone for STOT RE 2; H373 (muscle). One of them suggested to include also the liver, another to include the thyroid as a target organ whereas another considered the blood to be of specific concern. Concerning the **thyroid**, the DS responded that effects were observed in males only and were reversible without a clear relationship to the treatment. In the absence of clear signs of thyroid toxicity within the range for classification in other repeated dose toxicity studies, the DS decided not to include the thyroid as a specific target organ. Regarding the **blood**, the MSCA reported that the decrease in Hb was associated with brown pigmentation (minimal) in liver Kupffer cells of females (which may be hemosiderin deposition) and that other haematology effects were noted (decreased MCV, RBC and Hct). The DS responded that these effects were only observed in the 90-day dog study and the decrease in Hb was only (marginally) over 20 % at day 56 (20.7 % decrease). Other blood parameters were not significantly changed or were also reversible. The DS further clarified that the pigmentation in liver Kupffer cells was observed in high dose females only and it is not clear if this effect (as well as the Hb level) is specific to blood toxicity. For the **liver**, one MSCA highlighted that hepatotoxicity of flupyradifurone has been observed in three different species

(rat, mouse and dog) and submitted an attachment during the public consultation. According to the MSCA, effects included absolute and relative liver weight gain, clinical chemistry findings (decrease of total bilirubin and glucose concentration and increase of triglycerides, urea, creatinine, alanine aminotransferase and alanine phosphatase) and observations of enlarged liver, centrilobular hepatocellular hypertrophy and prominent lobulation. The DS responded that the effects observed seem reversible and more consistent with an adaptive response and not sufficiently severe, rather than signs of marked organ dysfunction.

Assessment and comparison with the classification criteria

There are no human data available with respect to repeated dose toxicity. However, the specific organ toxicity of flupyradifurone has been well tested in animals (rats, mice and dogs) via the oral route. There is also one 28-day dermal rat study. Due to the outcome of the acute inhalation toxicity study and the low vapour pressure of flupyradifurone (9.1×10^{-7} Pa at 20 °C) an inhalation study was not performed.

In addition to the studies considered by the DS for the evaluation of this endpoint, RAC also assessed a 28-day immunotoxicity and a 90-day neurotoxicity study (both performed in rats) as well as two long-term studies performed in rats and mice, two reproductive rat studies, two developmental toxicity studies performed in rats and rabbits and a developmental neurotoxicity rat study.

Repeated-dose toxicity studies

Based on the available repeated-dose toxicity studies, the main effects of concern were effects in the muscles (e.g. dogs). In addition, in different species effects on body weight were observed. A table which includes all relevant studies considered for this endpoint is presented below. Supplemental information (in depth analyses by RAC) is presented in the background document.

Summary of effects in repeated dose toxicity studies relevant for STOT RE

Table: *Summary of the described study reports including NOAEL and LOAEL*

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day#	Reported effects relevant for STOT RE 2
28-day	rat	oral, gavage	75[1]	**200**	Changes in biochemical parameters, increased liver weight, enlarged liver, prominent lobulation, hepatocellular hypertrophy, follicular cell hypertrophy
28-day	rat	oral, diet	33.6[1]	385	Changes in biochemical parameters, increased relative liver and thyroid weight, prominent lobulation of the liver, hepatocellular hypertrophy, follicular cell hypertrophy
28-day	mouse	oral, diet	98[1]	-	
28-day	dog	oral, diet	M:62; F: 77[1]	**118**	Overall body weight loss and reduced body weight gain
28-day	rat	dermal	500	-	
28-day immunotoxicity study	rat	oral, diet	50	**230**	Body weight loss and reduced body weight gain, decreased food consumption
90-day	rat	oral, diet	6.0	**30.2**	Changes in biochemical parameters, increased liver and thyroid weight, enlarged liver, dark thyroid gland, hepatocellular hypertrophy, follicular cell hypertrophy
90-day	mouse	oral, diet	81	407	Reduced body weight, changes in biochemical parameters, increased liver weight, decreased kidney weight, pale liver,

					hepatocellular vacuolation, loss of normal multifocal/diffuse cortical epithelial vacuolation
90-day	dog	oral, diet	12	**33**	Reduced body weight gain, changes in biochemical parameters, significant increased kidney weight, myofiber atrophy/degeneration
90-day neurotoxicity	rat	oral, diet	M: 29.4 F: 34.8	M: 143 F: 173	Reduced body weight gain and food consumption. Enlarged liver (F: 4/6)
1-year	dog	oral, diet	7.8	**28.1**	Degeneration of skeletal muscle and other muscle (biceps femoris) degeneration
2-year	rat	oral, diet	15.8/18.5 (104/52 weeks)	-	Effects in the liver and to a less extend effects in the thyroid and lung
2-year	mouse	oral, diet	M: 45; F: 53	-	Effects in the liver and the kidney
One-generation	Rat	oral, diet	F and pups: 17.5	**F: 60.0** **Pups: 60.9**	Reduced body weight, reduced body weight gain, alterations in food consumption during premating and decreased spleen weights in females. Pups: Maternal effects leading to secondarily-mediated effects on body weight, body weight gain and changes in brain weight
Two-generation	rat	oral, diet	P: 6.4	**P: 32**	Reduced body weight, reduced body weight gain
Dev. toxicity	rat	oral, gavage	F and pubs: 50	-	F: Reduced body weight gain and reduced food consumption, increased absolute liver weight
Dev. toxicity	rabbit	oral, gavage	F: 15 Pups: 40	-	Reduced body weight gain, reduced food consumption
Dev. neurotoxicity	rat	oral, diet	F: 42.4 Offspring: 42.4	F: 102 Offspring: 102	F: decreased body weight gain. increased auditory startle habituation Offspring: decreased body weight gain, increased startle amplitude in females only on PND 60 and increased motor and locomotor activity on PND 13 in males only

[1] Dose range studies; # values in bold are within guidance values and therefore relevant for classification

According to the CLP criteria, substances are classified in STOT RE 2 based on evidence from studies in experimental animals that can be presumed to have the potential to be harmful to human health following repeated exposure. For classification in Category 2, animal data in which significant toxic effects of relevance to human health were observed at generally moderate exposure concentrations. The CLP Regulation provides a 'guidance value' of 10-100 mg/kg bw/day from a 90-day study to assist classification in Category 2. For a 28-day study this guidance value is ≤ 300 mg/kg bw/day.

Summing up on the myofiber atrophy/degeneration, RAC considers that the **effects in muscles** observed in the long-term dog studies are rather severe since atrophy and necrosis were observed in the 1-year dog study. In addition, these effects do not seem to be a secondary effect due to weight loss as in the 1-year dog study weight loss did not occur. RAC concludes that classification for **STOT RE 2 is warranted** as myofiber atrophy/degeneration (irreversible effect) was observed in both sexes at dose levels much lower than 100 mg/kg bw/day in a 90-day dog study, supported by the same findings in a 1-year dog study at 28.1/28.2 mg/kg bw/day (at slightly higher doses than the extrapolated CLP-Regulation value for STOT RE 2 which is ≤ 25 mg/kg bw/day). RAC points out that the values and ranges stated in the legislation are not intended as strict demarcation values. Besides, in the 90-day study in dog, clear changes in clinical chemistry (at the 2-month evaluation) indicative of muscle toxicity (creatinine

phosphokinase (CK) and aspartate aminotransferase) support the conclusion that the muscle effects are sufficiently severe for classification although no CK isoenzyme analysis was performed. In addition, as no underlying mode of action is demonstrated, the relevance for humans cannot be excluded.

Regarding the liver, although increased absolute and relative liver weight, clinical chemistry findings (e.g. decrease of total bilirubin and glucose concentration and increase of triglycerides, alanine aminotransferase and alanine phosphatase) as well as centrilobular or diffuse hepatocellular hypertrophy and prominent lobulation were observed in three different species (in rats and dogs at dose levels relevant for classification as STOT RE 2) and in both males and females, RAC considers these effects to be non-specific (adaptive) effects and not as indications of a marked organ dysfunction.

The results of the two chronic toxicity/carcinogenicity studies in rats and mice provide further support that the effects in the liver might not be severe enough to consider the liver as a target organ. Indeed, in the carcinogenicity study in rats, 1/60 males had marked eosinophilic foci at the top dose, 1/60 male rats had a marked periportal diffuse macrovacuolation at the top dose and 1/60 male rats had a marked periportal diffuse macrovacuolation at the mid dose. In the lung, 1/60 females had a marked focal foamy macrophage. All the other findings (in the liver, the lung and the thyroid) were minimal to moderate with a slight treatment-related increase in severity in both males and females. In mice, all findings were minimal to moderate with the exception of 5/50 male mice with a marked centrilobular diffuse vacuolation. All the other findings (in the liver and the kidney) were minimal to moderate with a slight treatment-related increase in severity in both males and females.

Effects on the **thyroid** which were clearly within the values according to Regulation (EC) No 1272/200 for STOT RE consisted in minimal follicular cell hypertrophy observed at 200 mg/kg bw/day in males in a 28-day rat study and in enlarged thyroid glands without any histopathological changes in the 28-day dog study (in males at 118 mg/kg bw/day and in females at 131 mg/kg bw/day). However, RAC does not consider these effects significant enough to warrant classification as there is no evidence of organ dysfunction.

Based on several independent studies in rats and rabbits, RAC concludes that flupyradifurone significantly affects the **body weight and (corrected) body weight gain** of parental animals and offspring that could not be counterbalanced by a significant increase in food consumption in the one- and two-generation studies. Significant body weight changes were observed well within the guidance value for STOT RE 2 (equivalent to a 90-day rat study: $10 < C \leq 100$ mg/kg bw/day). However, these effects are not considered severe enough to justify a classification for specific organ toxicity.

Other effects like the effects on the **blood system** (e.g. decreased Hb, Hct, MCV in the high dose group in the 90-day-study in dogs) are not severe enough to justify a classification with the blood as a target organ.

Overall, RAC concludes that, based on irreversible myofiber atrophy/degeneration in both sexes in dogs at doses within the relevant guidance values in the CLP Regulation, flupyradifurone should be classified as **STOT RE 2; H373: "May cause damage to organs (muscle) through prolonged or repeated exposure"**. As there is only one repeated dermal dose toxicity study in rats with some uncertainty with regard to the highest dose tested and as there are no studies performed via the inhalation route, RAC cannot exclude the possibility that the substance can exert toxicity by these routes. RAC therefore considers that the route should not be included in the hazard statement.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter did not propose a classification for germ cell mutagenicity as neither in the *in vitro* studies using bacteria and mammalian cells nor in the *in vivo* studies was any mutagenic potency of flupyradifurone observed.

Comments received during public consultation

No specific comments were received for this endpoint.

Assessment and comparison with the classification criteria

The mutagenic and DNA damaging potential of flupyradifurone was studied ***in vitro*** in two GLP-compliant Ames tests, similar to OECD guideline 471, conducted with flupyradifurone (purity 96.2 %/97.2 %), in a mammalian cell cytogenetic assay test and in a mammalian cell gene mutation test, both OECD- and CLP-compliant, conducted with flupyradifurone (purity 96.2 %) and ***in vivo*** in two mouse OECD- and CLP-compliant micronucleus tests, conducted with flupyradifurone (purity 96.2 %/97.2 %).

There was neither an increase in the number of revertant colonies in the Ames tests, nor any clastogenicity or mutagenic effects *in vitro* nor in the two *in vivo* micronucleus assays in mice.

RAC therefore agrees with the conclusion of the DS that classification of flupyradifurone for germ cell mutagenicity is not warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of flupyradifurone has been investigated in two dietary GLP-compliant carcinogenicity studies, one in rats (similar to OECD TG 453) and one in mice (similar to OECD TG 451), conducted with flupyradifurone (purity 96.2 %).

There were no human data available.

None of the studies conducted in rats and mice revealed any evidence of carcinogenic effect. Therefore the DS concluded that no classification for carcinogenicity is warranted.

Comments received during public consultation

There were no specific comments for this endpoint.

Assessment and comparison with the classification criteria

The both long-term studies in rats and mice which did not show any neoplastic leasions at any dose level. Non-neoplastic effects are described in detail in the section *"RAC evaluation of specific target organ toxicity - repeated exposure*" and in the background document.

Therefore, RAC agrees with the DS that **no classification for carcinogenicity is warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Effects on sexual function and fertility

The DS proposed classification for reproductive toxicity Category 2 (Repr. 2; H360) based on the results of a two-generation study in rats. In this study, a reduced number of estrous cycles of the F1 high dosed females in connection with a reduced number of implantation sites and pups were observed. These effects were found in dams with reduced body weight of 16 % in the pre-mating period.

Such effects were not found in parental generation which had a 5 % lower exposure during the pre-mating period although the body weights of these animals were also reduced compared to the controls, but only by 10 %.

In the range-finding one-generation test with even higher exposure levels no effects on oestrous cycle, implantation sites and pups were observed. Therefore the DS was not sure where these effects in the F1 females were on fertility which were only observed in the second generation or whether these effects are developmental effects due to in utero exposure but only observed during mating.

As the effects described may be secondary to reduced maternal body weight, the DS presented the findings from five studies investigating the impacts of reduced body weight on estrous length, number of corpora lutea and implantation rate. Brief descriptions of these studies are provided below:

Chapin (1993): Feed restriction in Sprague-Dawley rats led to reductions in body weight by 30 % which were accompanied by in an increase of estrous cycle length by 22 % at week 8-9, in a significant reduction of the number of corpora lutea/dam and in a non-significant reduction of implants/dam.

Terry (2005): A feed restriction dependent effect on estrous cycle length and corpora lutea in Sprague-Dawley rats, limited to rats still gaining weight as observed in the study by Milius (2011) using flupyradifurone.

Tropp (2001): The effect of food reduction on rats varied between strains.

Copper (1967): Feed restriction led to a dose-dependent increase of estrous cycle length in Wistar rats but there was no clear relationship between body weight and estrous length.

Carney *et al*. (2004): In the F1 generation a decrease in the number of estrous cycles and a slight decrease (which was well within historical control ranges) in litter sizes after 50 % feed restriction were observed whereas after 30 % feed restriction no such effects were described.

According to the DS, it is clear that reduced body weight results in an increase in estrous cycle length and in a reduction in the number of developing eggs but it is not clear whether the observed effects on the F1 females are secondary to the reduced body weights. Besides, it is not clear whether the described effects should be considered an effect on fertility or on development (due to *in utero* exposure). Therefore no specification on fertility or development is suggested resulting in a proposal to classify flupyradifurone for Repr. 2; H361. Regarding additional *in vitro* studies performed to determine whether flupyradifurone possesses endocrine disrupting properties, the DS considered the estrogen receptor transactivation assay as negative, the androgen receptor binding assay as equivocal and the steroidogenesis assay as positive. Effects observed in these studies occurred only at relatively high concentrations, but were reproducible in repeated runs. No metabolites of flupyradifurone were tested. Therefore, the DS concluded that based on these results, it can neither be confirmed nor excluded that flupyradifurone is an endocrine disruptor.

Developmental effects

According to the DS, no classification for developmental toxicity would be warranted (based solely on the outcome of the three developmental toxicity GLP-compliant gavage studies) as the limited effects on reduced pup weights observed in the generation studies at dose levels also inducing reduced maternal weight are considered to be limited effects in presence of maternal toxicity. Further, the effects observed in the developmental studies on rats and rabbits and in the developmental neurotoxicity rat study were also related to maternal toxicity (observed in the high dose groups).

Comments received during public consultation

Two industry comments were of the opinion that flupyradifurone does not meet the criteria to classify flupyradifurone for Repr. 2. They point out that the limited effects on reproductive parameters are clearly associated with reduced maternal body weight. After re-evaluation of the estrous cycle data using the method of Goldman and additional statistical analyses, industry confirmed that the marginal variations of the parameters observed at the high dose correlate well with reduced body weight. Therefore in absence of other effects, industry concluded that no classification for reproductive/developmental toxicity is warranted. Industry also commented on the results of additional *in vitro* endocrine disruptor assays in relation to the effects observed in the two-generation study in rats. According to them, there is no evidence of estrogen receptor activation by flupyradifurone. Further, the high-concentration effects in the androgen receptor binding and steroidogenesis assays were equivocal and inconsistent with an androgen antagonist mode of action. Industry also pointed out that no effects were observed consistent with a disruption of the hypothalamic-pituitary-gonadal mode of action. Industry also submitted qualitative comparisons of flupyradifurone with other substances. Overall, Industry concluded on no evidence of estrogen receptor activation and that the high-concentration effects in the androgen receptor binding and steroidogenesis assays are equivocal.

Four MSCAs, however, agreed with the proposed classification as Repr. 2; H361 without a specification (f or d letters) as it is not clear if the concern on reproductive toxicity may be related to fertility or development. In relation to the weight of evidence in favour of classification as Repr. 2, one MSCA pointed towards (equivocal) indications of endocrine activity *in vitro*.

Assessment and comparison with the classification criteria

The assessment of reproductive toxicology is based on a one-generation dose range-finding study, a two-generation study, three developmental toxicity studies, one developmental neurotoxicity study and three endocrine disruptions assays.

Assessment of sexual function and fertility

A one-generation dose range-finding study, a two-generation study and a developmental neurotoxicity study, all conducted in rats with flupyradifurone (purity 96.2 %), were available.

A **one-generation** GLP-compliant **dose range-finding** pilot study was performed in 10 Wistar rats/sex/dose in order to determine appropriate dietary levels for a two-generation study; the substance was administered continuously (*ad libitum*) at nominal dietary concentrations of 0, 200 (M: 14.5 mg/kg bw/day; F: 17.5 (premating), 15.8 (gestation), 17.5 mg/kg bw/day (lactation)), 700 (M: 50.1 mg/kg bw/day; F: 60.0 (premating), 48.8 (gestation), 60.9 mg/kg bw/day (lactation)), and 2 000 ppm (M: 147.5 mg/kg bw/day; F: 168.9 (premating), 164.4 (gestation), 182.3 mg/kg bw/day (lactation)).

There were no treatment-related deaths or clinical signs. In females, declines in body weight and

in body weight gain (non-statistically significant at the mid-dose and statistically significant at the high dose) were observed as well as significant decreases in absolute and relative spleen weight at the high dose and decreases in absolute but increases in relative brain weight. In males, very slightly decreased body weight gain and decreased food consumption during premating in the high dose group were found as well as increases in the liver weight in the mid- and high-dose group and increases in absolute and relative spleen weights at the high dose. Declines in absolute pup weight in both sexes (reaching statistical significance for females) were observed in the 700 (60.9 mg/kg bw/day) and 2 000 (182.3 mg/kg bw/day) ppm dietary groups, beginning on PND 14 and continuing to PND 21 as well as declines in body weight gain. There were no substance-related gross necropsy findings at any dietary level tested.

In a GLP-compliant **two-generation study**, similar to OECD TG 416, Wistar rats were exposed to a nominal dietary concentration of 0, 100 (6.4 to 7.8 mg/kg bw), 500 (32.0 to 42.2 mg/kg bw) and 1 800 (117.4 to 168.8 mg/kg bw) ppm.

Statistically significant decreases in body weight during premating, gestation and lactation were observed in parenteral females (at the highest dose level), in F1 females (at mid dose (< 10 %) and at high dose) and in high dose F1 males throughout exposure. Decreases in body weight gain were found in high-dose parenteral (non-significant) and mid- and high-dose F1 females during premating (non-significant) and in high-dose F1 females during gestation (significant) as well as in mid- and high-dose F1 males during premating (significant). During gestation, in high-dose F1 animals of both sexes increased food consumption was observed (statistically significant (g/kg/day), non-significant (g/animal/day)) whereas during pre-mating the food consumption (g/animal/day) in high-dose parental and F1 females was statistically significant decreased.

In high-dose F1 females, a statistically significant decrease (2.9 vs. 3.5 in controls) in the number of oestrous cycles (parallel to significant weight loss), a concomitant non-significant increase in cycle length (4.4. vs. 4.0 in controls) and a significant decline in the total number of implantation sites (-17 %) without a clear dose-response relationship was noted.

Historical control data were not provided. Therefore, the decrease in the number of oestrous cycles, the increase in the cycle length and the decline in the total number of implantation sites cannot be related to previous study results.

Table: *Estrous cycle length and periodicity*

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.1)	3.7 (0.1)	3.4 (0.2)	3.4 (0.1)
Estrous Cycle Length (S.E.)	4.4 (0.3)	4.3 (0.1)	4.3 (0.2)	4.3 (0.1)
F_1-Generation				
Number of Estrous Cycles (S.E.)	3.5 (0.2)	3.3 (0.2)	3.3 (0.2)	2.9* (0.2)
Estrous Cycle Length (S.E.)	4.0 (0.2)	4.1 (0.1)	4.4 (0.2)	4.4 (0.1)

* Statistically different from control, $p \leq 0.05$ Data taken from Table 6 in the study report.

Table: *Reproductive performance*

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P-Generation – F_1-Offspring				
Number Cohoused	30	30	30	30
Number Mated	30	29	29	30
Number of Animals Delivered	29	27	28	28
Number of Animals with Implants	29	27	28	28
Mating Index	100.0	96.7	96.7	100.0
Fertility Index	96.7	93.1	96.6	93.3
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	3.4 (0.67) 3.0	3.3 (0.59) 3.0	3.2 (0.52) 3.0	3.1 (0.59) 3.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.1 (0.11) 22.0	22.1 (0.12) 22.0	22.1 (0.12) 22.0	22.0 (0.12) 22.0
Total number of implantation sites (Median)	311 (11.0)	285 (11.0)	298 (10.5)	289 (10.0)
F_1-Generation – F_2-Offspring				
Number Cohoused	30	30	30	30
Number Mated	29	30	29	30
Number of Animals Delivered	27	28	28	29
Number of Animals with Implants	27	28	28	29
Mating Index	96.7	100.0	96.7	100.0
Fertility Index	93.1	93.3	96.6	96.7
Gestation Index	100.0	100.0	100.0	100.0
Mean Number Days to Insemination (S.E.) Median	2.3 (0.23) 2.0	3.7 (0.45) 4.0**	2.6 (0.24) 2.5	2.1 (0.18) 2.0
Mean Gestation Length (days) (S.E.) Median Gestation Length (days)	22.0 (0.09) 22.0	22.1 (0.15) 22.0	21.9 (0.09) 22.0	22.0 (0.08) 22.0
Total number of implantation sites (Median)	305 (12.0)	314 (11.0)	323 (11.0)	281 (10.0**)

[a]Data obtained from Table 6 in the study report ** Statistically different from control, $p \leq 0.01$

Industry provided a qualitative evaluation of the individual relationship between body weight gain and the oestrous cycle. Their analysis concluded that animals that weighed less immediately prior to pairing were more likely to have fewer estrous cycles (relative to controls). This relationship was less apparent in the P0 generation than in the F1 females since the difference in bodyweight at the end of the pre-mating period (10 weeks) between the controls and high-dose animals in the P0 generation (10 %) was smaller compared to the F1 generation (15.9 %). Industry claimed that the difference in bodyweight reduction between the two generations is likely to be due to the increased duration of exposure of the F1 animals (i.e. through gestation, lactation and puberty) compared to the P0 animals. However, a relationship between body weight decrease and a decreased reproductive success is difficult to ascertain from the analysis provided by Industry. Besides, RAC supports the DS's opinion that as the original study was performed and analysed according to OECD TG 416, there is no reason to consider the Goldman *et al*. (2007) approach more appropriate for analysing the study than the approach outlined in the guideline.

With regard to offspring, no effects on the litter size, pup viability, sex ratio or lactation index were observed in the F1 pups. In the high-dose F2 pups, a significant decrease in the median litter size (outside the performing laboratory´s historical control range, according to the applicant) was noted while the mean litter size was not statistically significantly decreased. However, the decrease in the litter size paralleled the reduced body weight gain during gestation and a statistically significantly decreased number of implantation sites in the high-dose F1 females. Industry claimed a clear relationship between early gestation day (GD0) body weight and the

number of implantations for both the P0 and F1 generations. GD0 was selected in the analysis to exclude the influence of the litter size. According to this analysis (table below), a clear partitioning effect occurs at a body weight greater than 220 g (the median body weight used as the basis for the grouping) in the F1 generation data. Animals with larger bodyweights generally had more implantation sites, particularly in the control animals. Linear regression between implantation site and GD0 bodyweight in controls and treated animals at the top dose (P0 and F1) had a regression coefficient of R^2 = 96 % and 80 % respectively (data not shown). The linear regression values in F1 and P0 animals were not given in the absence of partitioning at the 220 g body weight cut-off. However, RAC agrees that bodyweight may have a significant influence over implantation site number, as indicated by the overall control values below (range: 9.6 to 12.2) and the correlation (data not shown). RAC notes that the influence of body weight (and probably of body weight gain during gestation) on implantation sites differed between controls and treated animals (slope of the linear regression line through the control data was 0.061 vs. 0.019, respectively). Therefore, RAC cannot exclude a direct relation to the treatment on implantation sites (primarily in F1 generation) in addition to a secondary influence of the body weight and body weight gain.

Table 2. Separation of Implantation data within groups by bodyweight

P_0 Generation	Median BW (g)	Grouping Criteria	Mean BW (g)	Mean # of Implants
Control	237.8	≥ median	258.4	11.7
		< median	219.7	9.6
1800 ppm	217.4	≥ median	229.1	10.4
	(91.4% of control)	< median	203.8	10.2
F_1 Generation	Median BW (g)	Grouping Criteria	Mean BW (g)	Mean # of Implants
Control	246.1	≥ median	257.4	12.2
		< median	229.9	10.3
1800 ppm	203.5	≥ median	199.7	9.9
	(82.8 % of control)	< median	191.2	9.6

Table: *Litter parameters*

Observation	Dose Group (ppm)			
	Control 0 ppm	LDT 100 ppm	MDT 500 ppm	HDT 1800 ppm
P Generation				
Total number of pups born	297	277	282	281
Number stillborn	0	2	0	0
Sex Ratio Day 0 (% male)	55.1	53.0	54.7	49.6
Mean litter size Day 0 Median	10.2 11.0	10.3 11.0	10.1 10.0	10.0 10.0
Birth index	95.0	97.1	93.3	97.4
Live birth index	100.0	99.0	100.0	100.0
Viability index	98.0	99.3	99.7	98.1
Lactation index	99.1	99.5	100.0	99.1
F_1-Generation				
Total number of pups born	291	300	311	266
Number stillborn	1	0	1	4
Sex Ratio Day 0 (% male)	51.6	50.6	50.6	47.7
Mean litter size Day 0 Median	10.8 11.0	10.7 11.0	11.1 10.5	9.2 **10.0***
Birth index	95.6	92.7	96.4	94.8
Live birth index	99.7	99.7	99.2	98.2
Viability index	99.3	99.7	99.4	97.2
Lactation index	99.1	100.0	100.0	98.7

[a] Data obtained from Table 6 and 20 in the study report * Statistically different from control, $p \leq 0.05$

Statistically significantly decreased body weight was observed in F1 litters at high dose and in F2 litters at mid dose (beginning at PND 14) and high dose (beginning at PND 7). Regarding the pups, Industry provided a similar analysis and concluded that the decrease in bodyweight at GD0 (> 15 %) had a strong influence on implantation sites, and consequently, the number of pups. In summary, for the three endpoints affected (number of oestrous cycles, number of implantation sites and number of pups), Industry argued that these were an effect of the body weight rather than of the treatment. RAC concurs that the reduced body weight gain results in an increase in oestrous length and in a reduction in the number of developing eggs but, as pointed out by the DS, it is not clear whether the observed effects on the F1 females are secondary to the reduced body weight. Besides, it is not clear whether the described effects should be considered an effect on fertility or on development (due to *in utero* exposure).

There was a significant delay in preputial separation and a non-significant delay in vaginal patency in the F1 pups, which might be secondary to effects of lower body weight as they were found in parallel with decreased pup weight. This effect was not specifically analysed by Industry but it was also considered related to decreased body weights exceeding the maximum tolerated dose. Reduced organ weights (thymus, spleen) in F1 and F2 litters of the high dosed group were also considered to be secondary to the decreased body weights. There were no treatment-related macroscopic abnormalities or histopathological findings in pups. Reduced brain weight was also observed but not consistently between studies. When observed, effects were not associated with e.g. clinical signs or histopathological findings. Summing up, some uncertainty remains whether the observed effects are only secondary effects to lower body weight.

Any other effects on reproductive performance (e.g. treatment-related effect on sperm parameters) were not found.

Assessment of development

Three developmental toxicity GLP-compliant gavage studies (two in Sprague-Dawley rats, one in New Zealand White rabbits) and one GLP-compliant developmental neurotoxicity study similar to OECD TG 426 were available. All these studies were performed with flupyradifurone (purity 96.2 %).

One of the rat studies was performed to provide additional information on maternal tolerability of flupyradifurone and to determine more precisely the NOAEL for maternal toxicity. In this study, concentrations of 0, 20 and 30 mg/kg bw/day were fed. The other study was similar to OECD TG 414, administering 0, 15, 50 and 150 mg/kg bw/day of flupyradifurone. The doses of the substance given to rabbits were 0, 7.5, 15 and 40 mg/kg bw/day.

In none of the three studies were treatment-related mortality or clinical signs observed. Treatment did not affect (e.g.) the pregnancy rate, the number of live foetuses or the number of implantation sites per dam, the percentages of pre and post implantation losses, the number of early and late resorptions, foetal deaths or the percentage of male foetuses.

In the OECD-compliant rat study, an increased incidence of a short costal cartilage at the high-dose and of parietal incomplete ossification without any dose response relationship was found. The increased incidence of unossified cervical centrum and of the incomplete ossification of the sternebrae was within the historical control data generated from 15 in-house Sprague-Dawley rat studies (2001-2008).

In rabbits, the skeletal variations (e.g. increased incidence of incomplete or unossified hyoid centrum, incomplete ossification of the 5^{th} and 6^{th} sternebrae, incomplete ossification of pubis) were within the in-house historical control data, generated from 14 developmental toxicity studies with New Zealand White rabbits.

In an oral developmental neurotoxicity study with Wistar rats the effects were limited to the highest dose. In maternal animals, decreased body weight gain was observed. In the offspring, not only decreased body weight gain (> 10 %) but also slightly increased motor and locomotor activity in males and slightly increased auditory startle habituation in females was observed. Flupyradifurone was given ad libitum (except during neurobehavioral testing) at concentrations of 0, 120, 500, 1 200 ppm with adjustment during lactation to maintain a more consistent dosage throughout the exposure period what resulted in an average mean daily intake of 0, 10.3, 42.4 and 102 mg/kg bw/day.

As a conclusion, since no teratogenic effects in the absence of maternal toxicity were observed in the studies in rats and rabbits, RAC agrees with the DS that based on the outcome of the developmental studies no classification for developmental toxicity is warranted.

In vitro endocrine disruption assays

In an Estrogen Receptor Transactivation Assay conducted in two independent runs with human cells (Cell Line Hela-9903) flupyradifurone did not act as an agonist of the estrogen receptor alpha (hERα).

The overall result of an Androgen Receptor Binding Assay, which was performed two times due to an error in the preparation of the stock solution of flupyradifurone as a result of which one testing was not considered valid, was categorised as equivocal.

In a Steroidogenesis Assay performed in three independent runs using human cell line H295R, a small but significant decrease in the production of testosterone in 2 out of 3 runs and a decrease in the production of estradiol in all three runs were observed at the highest test concentration (100 µM). Therefore, flupyradifurone is categorised as positive based upon the results of this assay.

Besides, it has to be pointed out that although the parent compound was only tested at rather high doses, the positive results in the *in vitro* Steroidogenesis Assay (which was reproducible) might be related to the observed effects. Disturbed steroidogenesis can interfere with the balance of androgens and estrogens, with possible effects on the oestrous cycle and follicular function. In addition, the *in vitro* tests were not performed with any metabolites of flupyradifurone although the substance is extensively metabolised.

Conclusion on reproductive toxicity

It is known that reduced body weight and body weight gain may result in an increase in estrous cycle length and in a reduction in the number of developing eggs, as described by the DS. There is also no doubt that maternal toxicity affects pup development (e.g. reduced body weight, reduced body weight gain, decreased oestrous cycle number, decreased litter size and decreased number of implantation sites in the F1 generation).

The reduced number of implantation sites and the slight decrease in median implantation sites (and thus decrease in litter size) in the F1 generation is considered a secondary effect due to decreased body weight and body weight gain. The effect was not observed in the P0 generation dams and the total number of implantation sites between the P0 and F1 generations in the control (311 and 305 in P0 and F1, respectively) and high-dose (289 and 281 in P0 and F1, respectively) are similar. Therefore, this slight decrease is likely due to the decreased body weight in the P0 females, which was observed as early as day 7 after initiation of exposure. No other effects were noted in female organ weights or histopathology in the two-generation reproduction study. Developmental toxicity studies in rats and rabbits did not show any significant decreases in the number of implantation sites in dams that showed a significant decrease in body weight, nor in any other changes in reproductive indices, tissue weights, or histopathology.

Although the decreased body weight and body weight gain at doses indicative of parental and offspring's toxicity may be an explanation for the reproductive effects observed, it is not clear whether all reproductive findings in the offspring are due to the parental decrease in body weight and body weight gain (e.g. delay in preputial separation and vaginal patency, reduced brain, thymus and spleen weights). It is doubtful whether the reduction in body weight is severe enough to cause an increase in estrous length (see Carney *et al*. (2004) and Chapin *et al.* (1993)). In the CLP Guidance (3.7.2.5.5), it is stated that the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects.

The slight effects on increased motor/locomotor activity and auditory startle habituation were noted at the same dose level in the offspring but they are considered by RAC as secondary to general toxicity. However, since the pesticidal mode of action of flupyradifurone is based on nicotinic acetylcholine receptor (nAChR) agonist property and no MoA studies have been provided, it cannot be excluded that neurotoxic effects observed in offspring are related to the above properties. Besides, a significant decrease of brain weight was consistently observed in offspring (on day 21) in the two-generation study. In the developmental neurotoxicity study, the high dosed males showed a decrease of brain weight of 5% on PND 75. However, since the decrease in brain weight cannot be related to the effects on nAChR and since there is no indication of a MoA, RAC does not consider these effects sufficient to support a classification for reproductive toxicity for flupyradifurone.

Taken together, although there are uncertainties on the extent of the maternal toxicity as well as on the mode of action of flupyradifurone to explain the reproductive effects (in particular the increased length of the estrous cycles and the decreased number of implantation sites in the F1 high dosed females), the data supports that they were due to maternal body weight reductions. In conclusion, **RAC does not consider classification for fertility or development warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify flupyadifurone as Aquatic Acute 1 and Aquatic Chronic 1 with an M-factor of 10 for both acute and chronic.

Degradation

A hydrolysis study conducted according to OECD TG 111 and in compliance with GLP at pH 4, 7 and 9 at 50 °C for 5 days, showed that flupyradifurone is hydrolytically stable.

The photodegradation of radio-labelled flupyradifurone in water was examined in three studies. In two of them, performed according to Japanese JMAFF Guideline OPPTS 835.2240, flupyradifurone decreased from 95-98 % of AR at time 0 to 17-8 % of AR at 35 and 28 hours of irradiation. The determined dissipation time DT_{50} was 14 hours. The major degradants included flupyradifurone-succinamide and flupyradifurone-azabicyclosuccinamide. In the third study, carried out according to an ECETOC method using polychromatic light, not more than 10 % degradation was measured after a maximum irradiation period of 500 minutes.

Ready biodegradability tests were not available.

Four different water/sediment studies were submitted in the CLH dossier: two aerobic studies (one with one radiolabel (GLP, Hellpointner and Unold, 2012) and one with two radiolabels (GLP, Menke and Unold, 2012)), an anaerobic water sediment study (GLP, Xu, 2012), and a water/sediment study for the metabolite difluoroacetic acid (GLP, Hellpointner and Unold, 2012).

In Hellpointner and Unold (2012), the aerobic transformation of [pyridine-2,6-^{14}C] flupyradifurone was studied in two different water/sediment systems (Honniger and Angler Weiher) for a maximum of 119 days in the dark at 20 ± 1 °C. The dissipation of flupyradifurone from the water phases was mainly characterised by rapid partitioning into the sediment. The estimated DT_{50} values were 193.1 and 246.9 days for the entire water/sediment test system from Honniger and Angler Weihe, respectively. The only major transformation products were carbon dioxide (6.8 % and 8.5 %) and formation of non-extractable ^{14}C-residues (NER, 25 % and 13.6 %).

In Menke and Unold (2012), the aerobic transformation of two test substances with radiolabels in different positions (FUR-^{14}C and ETH-^{14}C) was studied in two water/sediment systems, Honniger Weiher (HW) and Angler Weiher (AW) for a maximum of 120 days in the dark at 20 ± 2 °C. The dissipation of the substance from the water phase was mainly characterised by a fast removal into the sediment.

In the entire water/sediment systems, flupyradifurone was degraded slowly (Xu, 2012).

The estimated DT_{50} values were 208.2 and 202.4 days for FUR and ETH test substances from HW, and 246.1 and 285.0 for FUR and ETH flupyradifurone from AW. Difluoroacetic acid was observed as a degradation product of ETH flupyradifurone in the water phases and in the sediment extracts of both water/sediment systems (Hellpointner and Unold, 2012).

The studies are all reliable and they are proposed by the DS as key studies for classification.

A final water-sediment study was an outdoor microcosm study (DT_{50} = 92.7 days, Burns, 2012). This study is not used as a key study for classification and labelling purpose but it still shows a low rate of degradation consistent with the other studies. Additional soil simulation studies and the anaerobic water-sediment study show similarly low levels of degradation. These studies are not directly used for classification and labelling purposes, but are considered supportive studies.

Based on the information above, the DS concluded that the substance is not considered to be rapidly degradable.

Bioaccumulation

For flupyradifurone, there was an available measured log K_{OW} of 1.2 according to OECD TG 117 (HPLC method). There are no measured BCF data.

Based on this information, the DS concluded that the substance has a low bioaccumulation potential.

Aquatic Toxicity

The DS provided acute and chronic aquatic toxicity data for the three trophic levels: fish, invertebrate, algae and aquatic plants. There is one amphibian toxicity study (frogs), however, this is believed not to influence the classification and is therefore not discussed.

The following table summarises the relevant studies on aquatic toxicity, all considered acceptable by the DS. The key studies used for the classification are indicated in bold.

Method	Results	DS Remarks	Reference
Acute Aquatic Toxicity Studies			
Acute Fish Rainbow trout *(Oncorhynchus mykiss)* FIFRA 72-1, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 74.2 mg a.s./L m.m.	Static, stock solution in DMF	Matlock & Lam, 2010a
Acute Fish Fathead minnow *(Pimephales promelas)* FIFRA 72-1, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 70.5 mg a.s./L m.m.	static, stock solution in DMF	Matlock & Lam, 2010b
Acute Fish Common carp *(Cyprinus carpio)* FIFRA 72-1, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 100 mg a.s./L nominal	Static, measured concentration was 101-120 % of the nominal concentration, stock solution in DMF	Bruns, 2011a
Acute Fish Sheepshead minnow (*Cyprinodon variegatus*) FIFRA 72-3, OPPTS 850.1075, OECD TG 203	LC_{50}, 96 h > 83.9 mg a.s./L m.m	Static, stock solution in DMF	Banman & Lam, 2009b
Acute Aquatic Invertebrate *Daphnia magna* FIFRA72-2, OPPTS 850.1010, OECD TG 202	EC_{50}, 48 h > 77.6 mg a.s./L m.m.	Static, stock solution in DMF	Banman & Lam, 2009a
Key Study **Acute Aquatic Invertebrate 48 hours, *Chironomus riparius* OPPTS 850.1300, OECD TG 202**	**EC_{50}, 48 h = 0.0617 mg a.s./ L nominal**	Static, measured concentrations were 97-107 % of the nominal	Bruns, 2011e
Acute Aquatic Invertebrate Eastern oyster *(Crassostrea virginica)* OPPTS 850.1025	EC_{50}, 96 h > 29 mg a.s./L	Flow through, stock solution in DMF	Gallagher *et al.,* 2009a
Acute Aquatic Invertebrate 96 hours, Salt water mysid *(Americamysis bahia)* OPPTS 850.1035	EC_{50}, 96 h = 0.26 mg a.s./L	Static, acceptable for juveniles only	Gallagher *et al.*, 2009b
Short term Algae & Plants *Pseudokirchneriella subcapitata* FIFRA 123-2, OPPTS 850.5400, OECD TG 201 (2006)	E_rC_{50} & E_yC_{50}, 72 h > 80 mg a.s./L nominal	Static, stock solution in DMF, measured concentrations were 111-120 % of the nominal concentration	Banman & Lam, 2010
Short term Algae & Plants 7 days, Duckweed *(Lemna gibba G3)* FIFRA 123-2, OPPTS 850.4400, OECD TG 221 (2006)	Lowest EC_{50} > 67.7 mg a.s./L m.m.	Semi-static, stock solution in DMF	Banmann, 2010

Chronic Aquatic Toxicity Studies			
Long Term Fish ELS test, Fathead minnow (*Pimephales promelas*) FIFRA 72- 4, OPPTS 850.1400, OECD TG 210	NOEC, 35 days = 4.41 mg a.s./L m.m.	Flow through, stock solution in DMF	Matlock & Lam, 2011
Long term Aquatic Invertebrate *Daphnia magna* EPA 72-4, OPPTS 850.1300, EEC C.20, OECD TG 211	NOEC, 21 days = 3.2 mg a.s./L nominal	Semi-static, stock solution in DMF Measured concentrations were 102-117 % of the nominal concentration	Riebschläger, 2011
Key Study **Long term Aquatic Invertebrate** **28 days, *Chironomus riparius*** **OECD TG 219**	**NOEC, 28 days = 0.010 mg a.s. /L nominal = (0.0041 mg a.s. / L m.m.)** ***NOEC = 0.00681 mg/L (geom. mean measured)**	Static, stock solution in DMF, measured concentrations decline to a mean 41 % in overlying water, still NOEC based on nominal concentration!	Bruns, 2011g
Long term Aquatic Invertebrate Saltwater mysid *(Mysidopsis bahia)* OPPTS 850.1350	NOEC, 28 days = 0.0132 mg a.s./L m.m.	Flow through, stock solution in DMF	Claude *et al.*, 2011
Short term Algae & Plants 7 days, Duckweed *(Lemna gibba G3)* FIFRA 123-2, OPPTS 850.4400, OECD TG 221 (2006)	Lowest NOEC = 34.2 mg a.s./L m.m.	Semi-static, stock solution in DMF	Banmann, 2010

*value not provided in the CLH report, but agreed by DS in the response to public consultation, from updated DAR December 2014, V3-B9 part 1 (see also Section "Additional key elements").

Acute aquatic toxicity

Tests was provided for four different fish species: Rainbow trout (*Oncorhynchus mykiss*), Fathead minnow (*Pimephales promelas*), Common carp (*Cyprinus carpio*) and Sheepshead minnow (*Cyrpinodon variegatus*). All studies have an LC_{50} > 70.5 mg a.s./L (96 h, m.m.).

Four different invertebrate species were also tested for the acute aquatic toxicity: the cladoceran *Daphnia magna*, the midge *Chironomus riparius*, the marine species *Americamysis bahia* and the mollusc *Crassostrea virginica*. The most sensitive organism was *Chironomus riparius* with an EC_{50} = 0.0617 mg a.s./L (48 h, nominal), therefore this was proposed as the key study for classification. The *Chironomus* study was based on nominal concentrations (acceptable because measured concentrations were 97-107 % of the nominal). The next most sensitive species is the salt water mysid *Americamysis bahia*, with an EC_{50} = 0.26 mg a.s./L (96 h, m.m.). The study is accepted only for juveniles, because it is not reported that a preliminary test with juveniles and young adults has been conducted to determine the most sensitive life stage (as the guidance prescribes). However, this study is reliable and used as a supportive study for the proposed classification, as its EC_{50} lies close to the value found in the *Chironomus* study.

Finally, one short term algae toxicity test (E_rC_{50} > 80 mg a.s./L, 72 h, nominal) and one *Lemna gibba* test (EC_{50} > 67.7 mg a.s./L, 72 h, m.m.) were reported.

Chronic aquatic toxicity

There are five chronic aquatic toxicity studies available.

One is a fish study (*Fathead minnow*) with a NOEC = 4.41 mg a.s./L (35 days, m.m.).

There are three invertebrate studies with respectively a NOEC = 3.2 mg a.s./L (21 days, nominal) for *Daphnia magna*, a NOEC = 0.010 mg a.s./L (28 days, nominal) for *Chironomus riparius* and a NOEC = 0.0132 mg a.s./L (28 days, m.m.) for *Mysidopsis bahia*.

Finally, a *Lemna gibba* study is reported; lowest NOEC = 34.2 mg a.s./L (7 days, m.m.).

The above chronic invertebrate toxicity studies confirm that *Chironomus riparius* is the most sensitive organism tested and therefore it is used as a key study for classification. The test proposed in the CLH report was based on the 28 d NOEC = 0.010 mg a.s./L nominal concentrations, although the mean measured concentrations declined to 41 % in overlying water after 28 days, (see also Section: "Assessment and comparison with the classification criteria"). The next most sensitive species is *Mysidopsis bahia* with a measured NOEC = 0.0132 mg a.s./L.

Metabolite aquatic toxicity

Several studies were available from the DAR and reported in the CLH report, related to the following metabolites: 6-CAN (6-chloronicotinic acid), DFA, (difluoroacetate), flupyradifurone-succinamide (in surface water) and flupyradifurone-azabicyclosuccinamide (in sediment).

According to these tests, all metabolites are less toxic than flupyradifurone. The lowest reported short–term value with 6-CAN was an EC_{50} > 1 mg/L (48 h, nominal) for *Chironomus tentans.* The most sensitive long term organism resulted *Daphnia magna* with a NOEC value of 43.3 mg/L flupyradifurone-succinamide (21 days, nominal). None of these data influence the classification of flupyradifurone.

Comments received during public consultation

Five MSCAs commented on the environmental classification. Four of them agreed with the proposed environmental classification, one of them asked for additional information. MSCAs who have commented, recommended the use of the mean measured concentration for the chronic toxicity end point of the key study test. The NOEC value of the toxicity test with *Chironomus riparius* was 0.010 mg a.s./L (nominal), however, according to the updated DAR December 2014, V3-B9 part 1, it should be recalculate to 0.00681 mg/L (geom. mean measured). The DS agreed with the commenters, but points out that the updated measured concentrations do not influence the classification (0.001 < NOEC ≤ 0.01 mg/L).

In addition, the DS clarified that for the chronic toxicity test with *C. riparius*, the concentrations were measured in the overlying water and pore water (not on the sediment phase) and that it was unlikely that the experiment was performed in the dark.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider flupyradifurone as not rapidly degradable.

The substance is hydrolytically stable at environmentally relevant pH and not ultimately degraded to a level greater than 70 % over 28 days in water/sediment simulation studies.

Bioaccumulation

RAC agrees with DS that flupyradifurone has a low potential to bioaccumulate in aquatic organisms. The basis for this is that log K_{OW} value of 1.2 is below the cut-off value of 4.

Aquatic toxicity

The most sensitive organism was *Chironomus riparius* with an EC_{50} = 0.0681 mg a.s./L (48 h, nominal, immobility) and therefore this was proposed as the key study for classification. The *Chironomus* EC_{50} was based on nominal concentrations. The study itself is reliable and the

measured concentrations were 97-107 % of the nominal values, therefore it is acceptable that the EC_{50} is based on a nominal concentration. In conclusion, based on the EC_{50} = 0.0681 mg/L being in the range (0.01 < $L(E)C_{50}$ ≤ 0.1), **RAC agrees to classify flupyradifurone as Aquatic Acute 1; H400 with an M-factor 10**.

Chronic aquatic hazard

The lowest value reported was a NOEC of 0.010 mg/L (nominal concentration) for the invertebrate *Chironomus riparius*. In the CLH report, the DS highlighted that after 28 days the measured concentrations declined by an average of 41 % in the overlying water: the measured concentrations of flupyradifurone in the overlying water were in the range 85-110 % (mean 99 %), 37-83 % (mean 60 %) and 30-52 % (mean 41 %) of nominal, after 1 hour, 7 days and 28 days, respectively.

As already stated in the "Additional key elements" section, RAC agrees with the DS's final conclusion that the NOEC should be based on mean measured concentrations. Indeed, in the additional DAR report (December 2014) it is stated that "*during pesticide peer review meeting 124 in December 2014, it was agreed that the endpoints for Chironomus should be expressed in terms of mean measured concentration in the water column when the analytical measurement concentrations are outside of the range 80-120 % and when concentrations in the sediment are not available*".

Therefore, according to the above table B.9.2.2.1-03, reported as an update in the Public Consultation response, the DS concluded that for *Chironomus riparius*, the NOEC based on geometric mean measured concentrations is equal to 6.81 µg a.s./L. The initial proposal is however not affected, because this value is in the same range than the nominal value for classification as Aquatic Chronic 1 (NOEC ≤ 0.01 mg/L).

In conclusion, flupyradifurone is considered to be not rapidly degradable, and **RAC agrees to classify flupyradifurone as Aquatic Chronic 1; H410 with an M-factor 10** (0.001 < NOEC ≤ 0.01 mg/L).

Additional references

DAR (2014). Flupyradifurone updated DAR, Volume 3 B9, part I. December 2014.

Anonymous (2012). Local Lymph Node Assay in the Mouse. October 2012.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

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