Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Fluopyram

EC Number: NA
CAS Number: 658066-35-4

CLH-O-0000001412-86-46/F

Adopted

04 December 2014

4 December 2014

CLH-O-0000001412-86-46/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: Fluopyram
EC Number: -

CAS Number: 658066-35-4

The proposal was submitted by **Germany** and received by the RAC on **13 September 2013.**

In this opinion, all classifications and labelling are given in accordance with the CLP Regulation; the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer provided.

PROCESS FOR ADOPTION OF THE OPINION

Germany 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 **9 October 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **25 November 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Peter Hammer Soerensen**

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. The comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 December 2014**.

The RAC opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **Fluopyram** should be classified and labelled as follows:

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	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	fluopyram (ISO); N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide		658066-35-4	Carc. 2 Aquatic Chronic 2	H351 H411	GHS08 GHS09 Wng	H351 H411			
RAC opinion	TBD	fluopyram (ISO); N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide		658066-35-4	Aquatic Chronic 2	H411	GHS09 Wng	H411			
Resulting Annex VI entry if agreed by COM	TBD	fluopyram (ISO); N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide		658066-35-4	Aquatic Chronic 2	H411	GHS09 Wng	H411			

SCIENTIFIC GROUNDS FOR THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

Summaries of tests for flammability, explosivity and oxidising properties were tabulated in the CLH report and it was concluded that no classification for these hazard classes is warranted.

Comments received during public consultation

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

The substance did not reveal any physical hazard properties relevant for classification and RAC agrees with the view of the Dossier submitter (DS) that based on the data presented no classification is warranted.

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

One oral, one dermal and one inhalation acute toxicity study, all in rats, were included in the CLH report. After oral and dermal exposure, no deaths occurred and no clinical signs of toxicity, effects on weight gain or gross pathological findings were seen at the only tested dose of 2000 mg/kg bw. In the acute inhalation toxicity study no deaths occurred at the highest technical achievable concentration of 5.11 mg/L. All exposed animals showed clinical signs such as bradypnoea, laboured breathing patterns, reduced motility and body temperature, piloerection, ungroomed hair-coat and limpness. In a battery of reflex measurements one female showed reduced tonus and vertical grip strength and impaired righting response. All clinical signs were fully reversible within 1 day (males) or 5 days (females).

Comments received during public consultation

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

The substance induced no mortalities after either oral, dermal or inhalation exposure at concentrations at or above the guidance values for classification. RAC thus agrees with the DS that no classification for acute toxicity is warranted for any of the exposure routes.

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

Summary of the Dossier submitter's proposal

No toxicity to a specific organ in the absence of lethality was observed in acute oral, dermal or inhalation toxicity studies in animals. Additionally, no acute organ toxicity was observed in short-term or long-term studies.

Comments received during public consultation

No comments were received for this hazard class

Assessment and comparison with the classification criteria

No effects that could lead to classification as STOT SE where reported. RAC thus agrees with the DS that no classification for STOT SE is warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One skin irritation study with fluopyram was included in the CLH report. In this study no systemic reaction to the test substance was observed. None of the three animals showed any irritation reaction throughout the study and the mean score according to the Draize scale was always 0.

Comments received during public consultation

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

In the reported skin irritation study there were no indications of irritation or corrosion. RAC thus agrees with the DS that no classification for skin corrosion/irritation is warranted.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

One eye irritation study with fluopyram was included in the CLH report. In this study no systemic intolerance reaction to the test substance was observed. Ocular findings were confined to conjunctival redness in two out of three rabbits after 24 hours. These findings had disappeared one day later.

Comments received during public consultation

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

Only minor and reversible effects were seen in the reported eye corrosion/irritation study. RAC thus agrees with the DS that no classification for eye corrosion/irritation is warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

One local lymph node assay (LLNA) was presented in the dossier. No mortality or clinical signs of toxicity were observed during the study. In particular, no cutaneous reactions were observed at the application site. Fluopyram did not induce any changes in the local lymph node stimulation index.

Comments received during public consultation

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

No signs of sensitisation were seen in the LLNA study presented. RAC thus agrees with the DS that no classification for skin sensitisation is warranted.

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

Summary of the Dossier submitter's proposal

A number of repeated dose studies were presented in the dossier.

Repeated dose oral studies
Oral exposure 28- and 90-day studies were performed in dogs, rats and mice. Additionally, a 1-year study in dogs was summarised in the dossier.

Rat:
In both rat studies (28 and 90 days exposure), treatment-related effects were found in the liver, kidneys and thyroid glands, characterised mainly as organ weight changes and hypertrophy (liver and thyroid), as well as hepatic enzyme induction. Hyaline droplet nephropathy was seen in males in both studies.

In the 28-day study fluopyram was administered in the diet at 0, 50, 400 or 3200 ppm (0, 4.0/4.6, 31.0/36.1, 254/263 mg/kg bw for m/f). A dose-related slight increase in total cytochrome P-450, benzyloxyresorufin O-dealkylation (BROD) and O-dealkylation of 7-pentoxyresorufin (PROD) activities, indicative of CYP2B activity, was observed in both sexes.

In the 90-day study fluopyram was administered in the diet at 0, 50, 200, 1000 or 3200 ppm (0, 3.06/3.63, 12.5/14.6, 60.5/70.1 and 204/230 mg/kg bw for m/f). A dose-dependent increase in T3, T4 and TSH was found in both sexes as well as a higher mean prothrombin time at the highest dose in males. After a 1 month recovery period there was partial reversibility, although some parameters remained statistically significantly different from controls.

In the 90-day study treatment-related effects at 200 ppm (adaptive liver weight increase due to minimal hepatocellular hypertrophy in 2/10 males, associated with non-significant changes in clinical chemistry parameters) were seen but these were not considered adverse. Centrilobular hepatocellular hypertrophy was found in all male rats and 7/10 and 10/10 females animals at 1000 ppm and 3200 ppm, respectively, with dose-related increases in severity associated with significant changes in blood biochemistry. Additionally, periportal to midzonal hepatocellular macrovacuolation was observed in 6/10 and 5/10 females at the mid and high dose, respectively. Therefore, the NOAEL was set at 200 ppm for both males and females (12.5/14.6 mg/kg bw/d), based on pronounced organ weight gain, clinical chemistry and histopathological findings in liver, thyroid and kidneys at the next higher dose level of 1000 ppm (60.5/70.1 mg/kg bw/d).

Mouse:
In the 28-day study in mice fluopyram was administered at 0, 150, 1000 or 5000 ppm (0, 24.7/31.1, 162/197, 747/954 mg/kg bw for m/f) in the diet. In those animals surviving to terminal sacrifice (3/5 females in the highest dose group were killed prior to terminal sacrifice for humane reasons), treatment-related effects were seen in the liver (organ weight changes, hypertrophy, single cell necrosis) and as hypertrophy in the adrenal glands, but only in females.

The top dose level of 5000 ppm (747/954 mg/kg bw/d) clearly exceeded the MTD due to the overt toxicity.

Also in the 90-day study pronounced liver effects and changes in related clinical chemical parameters were observed. Liver weights were dose-relatedly increased at 150 and 1000 ppm (0, 5.4/6.8, 26.6/32.0, 188/216 mg/kg bw for m/f) (at the high dose in 5/10 males and 10/10 females) due to minimal to moderate hepatocellular hypertrophy in both sexes. Additionally, there was an increase in slight focal necrosis in 3/10 males and 6/10 females at 1000 ppm compared to the controls. Effects of treatment seen in the adrenal gland were increased organ weights (males) and slight cortical vacuolation in females. The NOAEL was set at 150 ppm for males and females (26.6/32.0 mg/kg bw/d) since the slight liver effects occurring at this dose were considered adaptive rather than toxic. The LOAEL was set at 1000 ppm for males and females (118/216 mg/kg bw/d).

Dog:
One 28-day (0, 30, 150 or 750 mg/kg bw/d), one 90-day (0, 800, 5000 or 10000/20000 ppm coresponding to 0, 28.5/32.9, 171/184 or 332/37 mg/kg bw for m/f) and one 1-year (0, 100, 400 or 2000 ppm coresponding to 0, 3.0/3.8, 13.2/14.4 or 67.6/66.1 mg/kg bw for m/f) study were summarized in the CLH report. In all three studies the main target organ was the liver. Increases in liver weight (28-day and 90-day study) and hepatocellular hypertrophy (seen as an adverse effect starting at 750 mg/kg bw/d in the 28-day study and in 4/4 males and females at 5000 and 20000/10000 ppm in the 90-day study) was reported in combination with related clinical chemistry changes such as increased alkaline phosphatase (AP) (seen in the 90-day and 1-year studies). In the 90-day study, hepatocellular single cell necrosis was also observed in 2/4 males and one of four females at 5000 ppm and 3/4 males at the highest dose.

Thymic involution and estrous cycle disturbance observed in the 90-day study were attributed to the body weight loss observed.

The NOAEL of 800 ppm as determined in the 90-day study was well below the LOAEL from the 1-year study. Due to the similarity of both studies with regard to test animals, experimental design and the observed changes, one overall NOAEL for subchronic toxicity in the dog of 800 ppm (28.5/32.9 mg/kg bw/d for males and females, respectively) was derived. The LOAEL was set at 2000 ppm for males and females (67.6/66.1 mg/kg bw/d) from the 1-year study.

Repeated dose dermal studies
One 28-day study performed in rats was summarised in the CLH report.
The animals were treated for a minimum of 6h/d, 5d/week with 0, 100, 300 or 1000 mg/kg bw/d. Substance related findings consisted of an increased prothrombin time (males) and cholesterol concentration (females). Additionally, increased liver weights associated with hepatic hypertrophy were observed in both sexes. Based on these findings, the systemic NOAEL was 300 mg/kg bw/d and the NOAEL for local effects was 1000 mg/kg bw/d, which was identical to the systemic LOAEL.

Overall conclusion
Significant liver effects were seen in rats, mice and dogs at moderate exposures after repeated dosing. These effects were not regarded by the DS to be severe enough for classification. Kidney effects (seen in male rats only) were regarded as rat specific and not relevant to humans. Also, for the described thyroid effects a rodent specific mechanism is assumed. Significant adrenal effects (seen in mice) were confined to high doses and were not regarded as relevant for a potential classification for STOT RE.

Comments received during public consultation

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

Potentially relevant effects were observed in repeated dose toxicity studies in liver, kidneys (rats only), thyroid (rats only) and adrenals (mice only). The thyroid and kidney effects are not considered to be of relevance to humans, due to a rodent specific mechanism, as discussed further in the carcinogenicity section. Substance related effects in the adrenal gland (cortical vacuolation) were seen in the mouse 90-day study (females only) and appeared only at 1000 ppm (216 mg/kg bw/d). Adrenal gland effects in other species were either not severe or appeared at concentrations above the guidance value in the CLP Regulation (for 28 day studies: $\leqslant$ 300 mg/kg bw/d). Therefore, findings in this organ are not considered relevant for classification. However, in the rat, macroscopic liver changes combined with statistically significant liver weight increases and microscopically minimal to slight hepatocellular effects were observed at doses relevant for classification. All effects showed a high tendency towards reversibility after a 1 month recovery period, although not all effects had fully reverted. In the dog, slight liver changes were seen at relevant doses. However, neither the effects in rats nor in dogs can be regarded as significant toxic effects. Therefore classification is not warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Two bacterial mutation assays, a chromosomal aberration and mutation assay in mammalian cells *in vitro* and one micronucleus test *in vivo,* were summarised in the CLH report. All tests were negative.

The DS thus argued that no classification for germ cell mutagenicity was warranted.

Comments received during public consultation

One Member State Competent Authority (MSCA) commented on mutagenicity, stating that a second *in vivo* test to investigate organ specific genotoxicity should have been conducted.

Assessment and comparison with the classification criteria

All mutagenicity studies presented were negative and of acceptable quality. RAC thus agrees with the DS that no classification for germ cell mutagenicity is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Two carcinogenicity studies were summarised in the CLH report, one in C57BL/6J mice with doses 0, 30 150 or 750 ppm (0, 4.2/5.3, 20.9/26.8, 105/129 mg/kg bw m/f)in the diet and one in Wistar rats with doses 0, 30, 150, 750(concentration changed from 750 ppm to 375 ppm from week 85 onwards) and 1500 ppm (0, 1.2/1.68, 6.9/8.6, 29/-, -/89 mg/kg bw in m/f) in the diet . Both studies were performed under GLP. Tumours were induced in the liver in female rats (adenomas and carcinomas) but not in male rats. It should be noted that male rats were considerably more sensitive to the substance than female rats and only one half to one quarter of the highest dose given to females could be given to the males. In this study mortality was high with a survival rate for males of 37.8 % in controls decreasing to 19.9% at the high dose and 68.3 % in control females decreasing to 51.0% at the high dose. At the high dose there was also a reduction in body weight in both sexes. In mice, follicular cell adenomas were seen in the thyroid in males but not in females. No difference in sensitivity between the sexes was seen in this study and there were no treatment-related deaths or clinical signs.

The DS argued that the mode of action (MoA) for the formation of the thyroid tumours was not relevant to humans. The DS stated, however, that there was no convincing evidence that the liver tumours observed were caused by a MoA not relevant to humans. The DS noted that Constitutive Androstane Receptor (CAR) activation, indicative of a MoA potentially not relevant to humans, is not shown and gene expression is different from phenobarbital (PB; known CAR activator). Further, although genotoxicity is excluded, no other potential mechanisms have been excluded (estrogen receptor (ER), gap junction intercellular communication (GJIC), aryl hydrocarbon receptor (AhR)). Since there are tumours only in one sex and one species in one experiment and the tumours where mainly benign, the DS considered classification as Carc. 2 – H351 as appropriate.

Comments received during public consultation

Two MSCAs supported the proposed classification. One industry representative and one individual argued that classification for carcinogenicity was not warranted. A substantial amount of new information was also submitted by Industry.

The new data included studies on CAR activation and some studies and argumentation on other MoAs and is summarised in the section "additional key elements" in the background document. Further details can be found in the RCOM.

Assessment and comparison with the classification criteria

Liver tumours

In the rat carcinogenicity study, liver cell carcinomas and adenomas were observed in female rats at the highest dose (1500 ppm, 89 mg/kg bw).

The mortality rate in control males after 24 months was twice as high as in control females (male control: 61.7%, high dose: 81.7%; female control: 31.7%, high dose: 50%). The reason is unclear. It can be argued that the toxicity is well above the MTD, as the mortality is between 15 and 32 % relative to the control, but given the high mortality also in the controls, this is less certain. No historical control data were provided for this study. However, data on Wistar rats from the same period of time is available from Charles River (Giknis and Clifford, 2011), indicating that 1 case of liver carcinoma was found in 40 studies, while adenomas were seen in 9 out of 40 studies with a frequenzy of between 1 and 11 (median 1).

One of the DS arguments for the Cat 2 classification proposal is the lack of CAR activation data. Data supporting CAR activation, including studies with knock out mice, were provided during PC. These studies reported the following:

- Activation of the CAR
  as shown in knock out mice (*Confidential study, 2013b)*, see Table 1 below;

- Specific CYP enzyme induction (CYP 2B family) including hypertrophy of liver
  shown in different oral repeated dose studies with female rats as gene transcription activation of phase I enzymes and as Cyp 450 isoenzyme profile as PROD activity and others;

- Increased hepatocellular proliferation in the rat
  shown in different oral studies with female rats treated for 3 to 28 days and as replicative DNA synthesis (S-phase) stimulation in *in vitro* study with primary rat hepatocytes (*Confidential study, 2013c);*

- Lack of hepatocellular proliferation (S-phase) in human
  shown in *in vitro* study with primary human hepatocyte (*Confidential study (2013d);*

- Reversibility of effects,
  28-day rat study (only with females) plus one month recovery showed recovery, although not complete;

Table 1 *Key event K/O mice study* (28-day oral male mouse study with WT (C5/BL/6J) and Pxr KO/Car KO strain)

Effects	WT mouse 1500 ppm Fluopyram	Pxr KO/Car KO mouse 1500 ppm Fluopyram
liver weight, - mean absolute - mean liver to BW ratio - mean liver to brain weight ratio	 + 66% +62% +66%	 +11% +9% +13%
Liver, enlarged in mice	14/15	-
Hepatocellular hypertrophy	15/15	-
Hepatocellular necrosis, min-slight	10/15	-
Hepatocytes, increased number of mitosis	3/15	-
Interstitial cell infiltration, focal	11/15	-
Increased PROD	151x	1.4x
Increased BQ	7.9x	1.7x
Increased Bil-GT	2.0x	-

Based on the data provided RAC considers that it has been demonstrated that a CAR mediated MoA contributes to the formation of liver tumours.

The possibility that other MoAs could be operating was also considered. Industry provided some experimental evidence against one of the MoAs and argumentation against several others (such as no structural similarities with estrogen and that the other MoAs would be seen in the K/O mice). RAC considers these arguments reasonable.

- Genetic toxicity can be ruled out based on the mutagenicity studies submitted.
- PPARα activation seems not likely due to lack of induction of Cyp4a1.
- Significant induction of the Cyp1a1 gene was reported in the CLH report. An assessment of microsomal proteins revealed a slight increase in mean EROD measurements in several repeated dose toxicity studies in the rat. This can be indicative of activation of AhR. However, for an AhR agonist the increase in EROD is normally considerably larger than found in the studies described in the CLH report. It can also be argued that activation of the AhR would be expected in both species and sexes, whereas liver tumours are seen only in one species and one sex. Also in the cells *in vitro* it would be expected to see proliferation if AhR activation would occur. It has to our knowledge not been shown that this applies to human hepatocytes, such as used in *Confidential study* (2013d) but it would be a reasonable assumption.
- There is no histopathological evidence for estrogens, statins, metals and infection mechanisms, and no structural dissimilarity to estrogens.

Table 2 Gene expression given as mean fold change relative to controls for female Wistar rats exposed to fluopyram for 3, 7, or 28 days.

28 days Dose (ppm)		**30**	**75**	**150**	**600**	**1500**
Associated receptor	Rat Genes	**3 days**				
Ahr	*Cyp1a1*	-1.2	1.1	1.7	**7.3**	**62.7**
Car	*Cyp2b1*	-1.6	1.1	**3.3**	**49.6**	**244.1**
Pxr	*Cyp3a3*	1.1	**1.5**	**2.6**	**8.2**	**21.5**
*Ppar*α	*Cyp4a1*	-1.1	NC	-1.1	NC	-1.3
Associated receptor	Rat Genes	**7 days**				
Ahr	*Cyp1a1*	1.4	1.8	**4.6**	**63.6**	**222.9**
Car	*Cyp2b1*	2.6	3.1	**14.4**	**326.5**	**1434.0**
Pxr	*Cyp3a3*	1.5	**1.9**	**3.6**	**12.4**	**28.6**
*Ppar*α	*Cyp4a1*	-2.1	-2.4	-2.3	-2.3	**-3.2**
Associated receptor	Rat Genes	**28 days**				
Ahr	*Cyp1a1*	1.8	2.3	**8.1**	**100.9**	**354.7**
Car	*Cyp2b1*	2.7	1.7	**10.9**	**212.5**	**1543.8**
Pxr	*Cyp3a3*	**1.8**	**3.7**	**5.3**	**17.1**	**50.4**
*Ppar*α	*Cyp4a1*	-1.2	-1.1	NC	-1.2	-1.4

Taken together, RAC considers the possible contribution of other MoAs sufficiently excluded.

Thyroid tumours:

In the carcinogenicity study in male mice, follicular cell adenoma was observed (7/50 investigated male mice) in the thyroid gland at the highest dose (750 ppm, 105 mg/kg bw/d). However, in female control mice 3 adenomas in 48 investigated thyroid glands were found. Historical control data were not reported.

For the discussion of the MoA for follicular cell adenomas, an extensive database was submitted during PC. The evidence seems to point to a CAR mediated (phenobarbital-like; PB) MoA. The key events documented by Industry during PC are:

- Activation of the CAR
  Shown in a study conducted with Pxr KO/Car KO mice (see Table 3 below).

- Induction of Phase II liver enzymes (e.g. UDPGT) including hypertrophy of liver. This was presented in different oral repeated dose studies with male mice as gene transcription activation of phase II enzymes and as enzyme activation such as UDPGT-Bil and UDGPT-T4. RAC notes, however, that the concentration of T4 was greatly reduced already 2 hours after administration of the substance (100 mg/kg bw/d), a time-point too early to be affected by induction (an increase in the amount) of UDPGT. Furthermore,

when the activity of UDPGT was measured after 28 daily administrations of fluopyram, a statistically significant increase was only observed at the two top dose levels (>102 mg/kg bw/d), whereas the concentration of T4 was decreased already from 5 mg/kg bw/d. It is thus not certain that UDPGT is a key event.

- Decrease of T4 (fast clearance), increase of TSH, T3 unaffected
  Shown in different oral repeated dose studies with male mice and i.v 125I-thyroxine clearance study.

- Increased thyroid cell proliferation
  Shown in a 28-day oral study with male mice.

Table 3 Key event K/O mice study

Effects	WT mouse 1500 ppm Fluopyram	Pxr KO/Car KO mouse 1500 ppm Fluopyram
Thyroid gland proliferation index (mean)	+ 2.6	-
Increased T4-GT	1.9x	-1.3x
Increased Bil-GT	2.0x	-
Tshb gene transcription in pituitary gland	+67%	-17%

*(*28-day oral male mouse study with WT (C5/BL/6J) and Pxr KO/Car KO strain)

Humans are often less sensitive to induction of thyroid tumours compared to rodents. It can, however, not be concluded that thyroid tumours in rodents are never relevant to humans. During PC, data were provided, including studies on knock-out mice, supporting CAR activation. Industry argued that:

"The main reasons for the difference in response between rodents and humans are as follows:

I. Rodents are more sensitive to thyroid hormone changes
II. Rodents have enhanced thyroid hormone elimination
III. Thyroxine binding globulin is major plasma protein in humans (which acts as a buffer), but not in rodents
IV. Consequence, the concentration of unbound T4 is greater in rodents than humans, resulting in greater susceptibility to metabolism and excretion and compensatory increase in thyroid follicular cell turnover, which over time can result in thyroid tumors."

This, together with the fact that the DS did not consider the thyroid tumours suitable for classification could lead to no classification. However, while the DS did not consider the thyroid tumours relevant for classification, their argumentation as to why not, was not extensive. Concerning non-relevance to humans humans, CLP guidance states for example that "*certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction (IARC, 1999; EU Specialised Experts, 1999)*". The MoA, it was argued by Industry, is indeed UGT mediated. However, the specialised experts excluded "*liver enzyme inducing agents such as PB*" from their recommendation. The specialised experts were not convinced that substances acting as liver enzyme inductors could significantly alter the levels of thyroid hormones. However, subsequent data shows that liver enzyme inductors may indeed alther the levels of thyroid hormones.

Although there is a plausible MoA suggested for induction of thyroid tumours via a CAR mediated MoA, the evidence of such tumours is scarce. There are studies where PB enhances the tumorigenic potency of genotoxic carcinogens (Hiasa *et al*, 1982, 1983: McClain *et al*, 1988). In these studies no induction of thyroid tumours were seen after treatment with PB alone, but the treatment period was short. Dellarco *et al.* (2006) reported on a substance inducing similar effects

on the thyroid as fluopyram and inducing tumours but although it was possibly via CAR activation, this was not shown.

Based on the data provided, RAC considers that in this case, it can be demonstrated that a CAR mediated MoA contributes to the formation of thyroid tumours.

- Other MoA have partly been excluded. As for liver tumours, genetic toxicity can be ruled out based on the mutagenicity studies submitted.
- Damage to Follicular cells - No histopathological evidence of overt cytotoxicity was observed in the thyroid in rodent studies
- Inhibition of thyroid peroxidase - Mechanistic studies using hog thyroid microsomes showed that fluopyram did not affect thyroid peroxidase
- Inhibition of T4 to T3 via indirect MoA is unlikely as serum levels of T3 were unchanged in rodent studies

Taken together, RAC considers the possible contribution of other MoAs in this case to be sufficiently excluded.

RAC concludes that it has beensufficiently demonstrated that the thyroid tumours induced by fluopyram are caused by a CAR mediated MoA. This MoA might give rise to thyroid tumours in rodents.

The relevance of this MoA based on enhancement of the metabolism and excretion of thyroid hormone by the liver, largely through induction of UGT enzymes, **is in the case of Fluopyram considered by RAC not to be relevant to humans.**

Conclusion on classification:

Thyroid tumours: RAC concludes that it is sufficiently well shown that the thyroid tumours induced by fluopyram were caused by a CAR mediated MoA. This MoA might give rise to thyroid tumours in rodents. The relevance of such an MoA based on enhancement of the metabolism and excretion of thyroid hormone by the liver, largely through induction of UGT enzymes, is consider by RAC not to be relevant to humans. RAC considers the possible contribution of other MoAs sufficiently excluded.

Liver tumours: Based on the data provided, RAC concludes that a CAR mediated MoA was contributing to the formation of liver tumours. This MoA gives rise to liver tumours in rodents, but there is evidence that the effects in human cells differ from rodent cells. RAC considers the possible contribution of other MoAs sufficiently excluded. RAC concludes that the CAR mediated MoA is assumed to be of no relevance to humans.

RAC concludes that no classification for carcinogenicity is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

One two-generation reproduction study in rats with the doses 0, 40, 220 or 1200 ppm in the diet and two teratogenicity studies, one performed in rats with the doses 0, 30, 150 or 450 mg/kg bw/d and one in rabbits with the doses 0, 10, 25 or 75 mg/kg bw/d, were summarised in the dossier.

Two-generation reproduction study
In the P- and F1-generations, increased liver weights associated with an increased incidence of centrilobular hypertrophy were observed. In parental males only, an increase in some clinical

chemistry parameters, increases in kidney weight (associated with an increased incidence of protein droplet nephropathy) and lymphocytic infiltration were found. In parental females (P and F1) some effects on body weight and blood parameters were observed. The parental systemic NOAEL was 220 ppm (14.5 mg/kg bw/d in males, 17.2 mg/kg bw/d in females).

The offspring NOAEL was 220 ppm (14.5 mg/kg bw/d) based on maternal effects leading to secondary effects on pup weight and pup weight gain. Also, a slight delay in preputial separation and decreases in spleen and thymus weights were noted.

No reproductive findings were observed up to the highest dose tested resulting in a reproductive NOAEL of 1200 ppm in both males and females (82.8/93.1 mg/kg bw/d).

Teratogenicity studies

Rat:

In the rat study, maternal toxicity in terms of lower maternal body weight gain and food consumption, higher liver weight and diffuse centrilobular hepatocellular hypertrophy was seen at mid and high dose levels. Developmental toxicity was observed at the high dose only in terms of slightly lower fetal body weight, and a slightly increased incidence of two visceral variations and malformations (thymic remnant present, ureter convoluted and/or dilated) and two skeletal variations and malformations (incomplete ossification of thoracic vertebrae and split thoracic vertebrae). Split cartilage in the thoracic centrum was found in 4/159 foetuses in the higest dose group (450 mg/kg bw/d) and in 1/160 foetuses in the lowest dose group (30 mg/kg bw/d) but these were within the historical control range. No findings were seen in the mid dose group. The split cartilage is identified as a malformation according to Solecki *et al.* (2001). However, the findings of all incidences were observed only in one single thoracic centrum and only in the highest dose group in presence of maternal toxicity. The maternal NOAEL was considered to be 30 mg/kg bw/d and the fetal NOAEL was considered to be 150 mg/kg bw/d.

Rabbit:

Maternal toxicity was observed in the form of reduced mean body weight gains and food consumption. Foetal toxicity was found at the same dose level, consisting of reduced body weight in both sexes. For both, the maternal and the foetal developmental toxicity, the NOEL was set at 25 mg/kg bw/d.

Overall conclusion

The appropriate animal studies showed no effects of the substance on reproduction or fertility. Additionally, in the summarised teratogenicity studies, only minor effects on development were seen. These effects consisted of slight changes in foetal body weights and visceral variations occurring at maternally toxic doses. Thus, classification as toxic to reproduction was not considered warranted by the DS.

Comments received during public consultation

One MSCA commented, suggesting that classification as Repr. 2 - H361d would be warranted based on the increased incidences of certain visceral and skeletal malformations/variations in rats and the malformation 'gall bladder absent' in rabbits.

The DS responded that the malformation 'gall bladder absent' occurring in rabbits was within the historical control range. Furthermore, there were no treatment-related skeletal malformations in the rat. The reported effects (incomplete ossification of thoracic vertebrae and split thoracic vertebrae) are considered to be variations and do not require classification for developmental toxicity.

Assessment and comparison with the classification criteria

The findings on reproductive toxicity were limited to absent gall bladder and skeletal variations (incomplete ossification). The gall bladder findings (two cases) were within the range of historical controls. This effect is thus not considered to be treatment related. Variants may, according to the

CLP regulation, not lead to classification if considered to be of low toxicological significance. RAC considers the effects not severe enough to warrant classification. RAC therefore agrees with the DS that no classification for reproductive toxicity is warranted. RAC regards the findings of split cartilage in thoracic centrum to be a malformation. Split cartilage occured in the highest dose group in association with maternal toxicity. No effects were seen in the middle dose group. One single incidence of split cartilage in the lowest dose group was within the historical control range. In all incidences the findings were observed only in one single thoracic centrum per animal.

RAC agrees that the effects were not severe enough to be a basis for classification and therefore agrees with the DS that classification as toxic to reproduction is not warranted.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Fluopyram currently has no a harmonised classification according to CLP Regulation. The dossier submitter (DS) proposes to classify the substance as Aquatic Chronic Category 2 (H411).

Degradation
A hydrolysis study according to OECD guideline 111 and in compliance with GLP was run at pH 4, 7 and 9 at 50 °C for 5 days. Fluopyram was hydrolytically stable under acidic, neutral and alkaline conditions. No major degradation products were detected at pH 4, 7 and 9. No half-lives could be calculated as the substance was stable to hydrolysis at all pH conditions.

The photodegradation of radio-labelled fluopyram in water at pH 7 was studied according to the EPA-FIFRA 161-2 guideline. The study, in compliance with GLP, was carried out at 25 °C with continuous artificial light for 13 days. Fluopyram undergoes limited transformation by photolytic processes to fluopyram-lactam (14% of applied radioactivity) and 14 unidentified transformation products (maximum 4.2% of applied radioactivity (AR). The DT_{50} value for fluopyram was found to be 23 days (mean) of continuous irradiation. Aquatic photolysis is not considered to be an important transformation route for fluopyram in the environment.

Photolytic degradation of fluopyram was also studied in natural water at 25 °C. The study was carried out according to the EPA-FIFRA 161-2 guideline and in compliance with GLP. Fluopyram phototransformed slowly with a DT_{50} of 21 days in natural water under continuous irradiation. No major transformation products were detected. A minor transformation product, fluopyram-lactam was observed at a maximum of 1.2% AR. Phototransformation would not be a principle route of transformation in natural waters.

No data on ready biodegradation are available.

A water/sediment simulation study, carried out according to OECD TG 308 and in compliance with GLP, was run for 120 days using two pond systems (Leverkusen, Germany and Lawrence, Kansas, U.S.A.). No major degradants were formed in either water/sediment systems. The maximum CO_2 evolved was 1,8% AR. The DT_{50} values of fluopyram in the entire system were estimated to be greater than 648 days in both sediment/water systems, which indicate that fluopyram is persistent in the aquatic environment. Aerobic biotransformation would not be an important transformation route of fluopyram in the aquatic environment.

Bioaccumulation
Fluopyram has a measured logK_{ow} of 3.3 (Method OECD 107, 20 °C).
The DS provided a bioaccumulation study on fluopyram. In this study (OECD TG 305) bluegill sunfish (*Lepomis Macrochirus*) were exposed to radio-labelled fluopyram over 28 days uptake phase and 14 days depuration phase. Two test concentrations of 6 and 60 µg/L were used.
Kinetic bioconcentration factor of 87.9 (whole fish, total radioactive residue-TRR) and 65.7 (whole fish, TRR) were determined from uptake and depuration rate constants. BCF values related to whole fish and TRR of 62.5 and 46.7 were obtained after normalization to 5 % lipid content of fish. Related to unchanged parent a low steady-state bioconcentration factor (BCF whole fish = 18; BCF whole fish normalized to 5% lipid content = 13) and a very rapid clearance half-life (1.8 to 3.4 days) were determined.

Based on these information the DS concludes that the fluopyram has a very low bioconcentration factor.

Aquatic toxicity

Several results on aquatic toxicity were provided for the three trophic levels (fish, aquatic invertebrates and algae/aquatic plants) other than for a sediment-dwelling organism.

Regarding toxicity to fish, two saltwater acute tests were available (*Cyprinodon variegatus* and *Lepomis macrochirus*). In both cases the LC_{50} values were above the practical limit of water solubility under test conditions and no effect were observed up to the highest measured concentration.

The DS provided a chronic test on *Pimephales promelas.* It is the key study for the chronic toxicity classification, with a **33-d NOEC=0.135 mg/L** (mean measured concentration).

Regarding toxicity to aquatic invertebrates, acute tests on *Daphnia magna* and *Americamysis bahia* showed EC_{50} values above the practical limit of water solubility under test conditions. No effect were observed up to the highest measured concentration for *D. magna*, while a 10% mortality was reported in the highest treatment group for *A. bahia.*

A 21-d semi-static chronic toxicity test on *D. magna* was provided, with a NOEC = 1.25 mg/L nominal (1.22 mg/L mean measured).

Regarding toxicity to algae and aquatic plants, the DS provided a 7-day acute toxicity study on *Lemna gibba* and two 96-h acute toxicity studies (the freshwater green alga *Pseudokirchneriella subcapitata* and the saltwater diatom *Skeletonema costatum*). While for the *Skeletonema costatum* the DS stated that no effect was observed up to the functional limit of solubility in the test system, for the green alga, a 72-h EC_{50} = 3.97 mg/L was reported based on effects on biomass and 8.9 mg/L based on growth rate.

The results obtained with the test on *Lemna gibba* are related to two endpoints: frond area based on yield and growth rate for frond number. The most sensitive endpoint was frond area based on yield (E_YC_{50} = 2.32 mg/L nominal; NOEC=0.256 mg/L nominal).The NOE_rC values, based on the growth rate, is 1.6 mg/L and the **E_rC_{50} = 2.51 mg/L** (nominal) based on growth rate is proposed as the key value for the acute toxicity classification.

Moreover the DS provided a 28-day static toxicity study on the sediment-dwelling organism *Chironomus riparius* according to OECD TG 219. The most sensitive endpoint was the emergence ratio. The reported NOEC was based on the nominal concentration of 1.39 mg/L and on the corresponding Time Weight Average (TWA) concentration of 0.525 mg/L. All the measurements were based on overlying water concentrations.

A summary of the most reliable ecotoxicity results were as follows in table 4 (the key studies for classification are highlighted in bold):

Table 4: Overview of the ecotoxicity data

Method	**Test organism**	**Test type**		**Results**		**remarks**
			Endpoint	**LC_{50}/EC_{50} [mg/L]**	**NOEC [mg/L]**	
Fish						
OECD 203 (rev. 1992), US EPA OPPTS 850.1075 (1996), FIFRA 72-3 (1982)	*C. variegatus*	96-h (static)	Mortality, LC_{50}	>0.98 mg/L * mm		
OECD 203 (rev. 1992), US EPA OPPTS 850.1075 (1996), FIFRA 72-1 (1982)	*L .macrochirus*	96-h (static)	Mortality, LC_{50}	>5.17 mg/L * mm		
EPA OPPTS 850.1400, OECD 210, SEP-EPA-560/6-82-002, ASTM E	*P. promelas*	**33-d (flow through) ELS**	**Length and morphological/ behavioral effects**		**0.135mg/L mm**	

1241-92						
Aquatic invertebrates						
OECD 202 (2004), EEC Directive 92/69/EEC (1992), JMAFF 12 Nousan No. 8147 (2000), FIFRA 72-2 (1982), EPA OPPTS 850.1010 (1996)	*D. magna*	48-h (static) 21-d (semi-static)	Immobility EC_{50} Offspring production, offspring behavior and parental body length,	>17 mg/L * mm	1.25 mg/L nom 1.22 mg/L mm	OECD TG 211, EEC Directiv C.20, US EPA 72-4, OPPTS 850.1300
EPA OPPTS 850.1035	*A. bahia*	96-h (flow through)	Mortality, EC_{50}	>0.51 mg/L * mm		
Aquatic algae and plants						
FIFRA Guideline 123-2 (1982), OPPTS Guideline 850.4500 (2006 draft), OECD Guideline 201 (2006)	*P. subcapitata*	72-h (static)	Biomass, E_bC_{50} Growth rate E_rC_{50} NOEC	3.97 mg/L mm 8.9 mg/L mm	1.46mg/L mm	
OECD Guideline 201 (2006), OPPTS 850.4500 (2006 draft), FIFRA 123-2 (1982)	*S. costatum*	96-h (static)	EC_{50}	>1.13 mg/L * mm	1.13mg/L mm	
OECD 221, EPA OPPTS 850.4400	*L. gibba*	7-d (static)	Fronds, E_yC_{50} **Growth rate E_rC_{50}** NOEC (frond area based on yield) NOErC (growth rate for frond number)	2.32 mg/L nom **2.51 mg/L nom**	0.256 mg/L Nom 1.6 mg/L nom	
Other aquatic organisms						
OECD 219	*C. riparius*	28-d (static,spiked water)	Emergence		0.525 mg/L mm (TWA)	

mm: mean measured concentrations
nom: nominal concentrations

* effect concentration above practical limit of water solubility under test conditions

Comments received during public consultation

Three MSs and one Industry representative contributed during public consultation. The MSCA stated general agreement with the proposed environmental classification and Industry made only editorial remarks.
One of MS suggested that the NOEC value for *Chironomus riparius* of 1.39 mg/L, based on nominal concentrations and validated in the peer review of the pesticide risk assessment of the active
substance fluopyram (EFSA 2013), should be considered more relevant than the NOEC value of 0.525 mg/L, which seemed to be based on TWA concentrations and not on mean measured concentrations. In addition, they asked to explain why the NOEC of *Lemna gibba* used in the CLH

report was based on the $NOEC_{yield}$ instead of the $NOEC_{rate}$. They concluded, however, that these comments will not change the conclusion of the classification proposal.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider fluopyram as not rapidly degradable. The substance is hydrolytically stable under acidic, neutral and alkaline conditions. In addition, aquatic photolysis is not considered to be an important transformation route for fluopyram in the environment. Although no studies on ready biodegradability according to OECD 301 are available, fluopyram is demonstrated to be not ultimately degraded to a level greater than 70% in a water/sediment simulation test.

Bioaccumulation

Based on experimental data, fluopyram has a logK_{ow} value of 3.3 (Method OECD 107, 20 °C). The measured BCF of 13 (normalized to 5 % lipid content) based on the parent compound showed that the bioaccumulation potential of fluopyram is low. Therefore, the BCF value is below the decisive CLP criterion (BCF≥500).

Aquatic toxicity

Acute toxicity data were available for all three trophic levels. The most sensitive aquatic species was *Lemna gibba*. The lowest and relevant reliable short-term aquatic toxicity result was **7d E_rC_{50} = 2.51 mg/L** (nominal concentration). This value is above the trigger for acute aquatic classification (1 mg/L), therefore no acute aquatic classification is necessary.

Chronic aquatic toxicity

Reliable and relevant long-term aquatic toxicity data are available for all three trophic levels. The lowest value is for *P. promelas*, with a **33 d NOEC=0.135 mg/L** (mean measured concentration). This value lies in the toxicity range of 0.1 < NOEC ⩽ 1.0 mg/L.

Conclusion on classification

Fluopyram is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation.
The lowest acute toxicity value falls above the trigger value of 1 mg/L and the lowest chronic toxicity value lies in the toxicity range of 0.1 < NOEC ⩽ 1.0 mg/L.

RAC concludes that fluopyram fulfils the CLP criteria for classification as Aquatic Chronic category 2 (H411).

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ANNEXES:

Annex 1 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 rapporteurs' comments (excl. confidential information).