Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLUOPYRAM (ISO); N-{2-[3-CHLORO-5-(TRIFLUOROMETHYL)PYRIDIN-2-YL]ETHYL}-2-(TRIFLUOROMETHYL)BENZAMIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: Fluopyram (ISO); N-{2-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS number: 658066-35-4
EC number: -
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Norway		MemberState	1
Comment received				
Norway would like to thank Germany for the proposal for harmonised classification and labeling of Fluopyram, CAS- no. 658066-35-4.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.11.2013	France		MemberState	2
Comment received				
FR supports the proposed classification for human health and the environment.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2013	United Kingdom		Individual	3
Comment received				
Expert Statement on Fluopyram (CLH Report, Version 2 of September 2013) **Dr Clifford R Elcombe, CXR Biosciences Ltd and University of Dundee** **Uploaded on ECHA homepage on Nov. 21, 2013**				

The Mode of Action (MoA) for the Non-Genotoxic Liver Carcinogenicity of Fluopyram in Rodents and Its Relevance to Human Hazard and Risk Assessment.

Introduction to Non-genotoxic Hepatocarcinogenesis

Several nongenotoxic mechanisms for hepatocarcinogenesis in rodents have been identified. These include sustained cytotoxicity, oxidative stress, oestrogenicity and nuclear hormone receptor activation (e.g. AHR, aryhydrocarbon hydroxylase receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor and PPARα, Peroxisome Proliferator Activated Receptor alpha). (Cohen *et al*., 2003; Cohen, 2010).

Despite different initial mechanisms, the common key event for all these pathways in the carcinogenic mode of action is the stimulation of hepatocellular S-phase and proliferation. In the absence of increased cell proliferation, carcinogenesis will not occur.

The CAR-activation MoA is typified with studies using phenobarbital. CAR activation involves hypertrophic and hyperplastic events. The hypertrophic events are characterised by the proliferation of smooth endoplasmic reticulum and associated enzyme induction (e.g. increases in CYP2B), while the hyperplasia is characterised by increased hepatocellular replicative DNA synthesis (S-phase) and cell proliferation. The sequence of key events in the phenobarbital-induced liver tumour MoA comprise activation of CAR followed by altered gene expression specific to CAR activation, increased cell proliferation, formation of altered hepatic foci and ultimately the development of liver tumours. Associative events in the MoA include altered epigenetic changes, induction of hepatic CYP2B enzymes, liver hypertrophy and decreased apoptosis (Elcombe *et al*., 2013; Figure 1). Similar events are initiated following the activation of a similar nuclear receptor, PXR. However, this is characterised by primarily, although not exclusively, the induction of CYP3A rather than CYP2B.

Studies using Car/Pxr knockout mice and CAR knockout rats have demonstrated that these events do not occur in the absence of functional CAR/PXR receptors. (Ross *et al*., 2010; Yamamoto *et al*., 2004; Chamberlain *et al*., 2014, in press).

Of importance are the observations that CAR activators ("phenobarbital-like" agents) induce CYP2B and, to a lesser extent, CYP3A, in rat, mouse and human primary cultures of hepatocytes. However, they **only** stimulate hepatocellular S-phase DNA synthesis in rodent hepatocytes and **not** in human hepatocytes (Lake, 2009; Elcombe *et al*., 2013).

This explains why evidence exists for phenobarbital-mediated enzyme induction in humans but **no** data exists to suggest that phenobarbital may elicit hyperplasia or liver tumours in humans (Lake, 2009; Elcombe *et al*., 2013). In fact, to the contrary, a recent review and reanalysis of the available phenobarbital epidemiology suggests that it does not increase the incidence of liver tumours (La Vecchia and Negri, 2013).

Considering all of these data, a recent Expert Panel (Elcombe *et al*., 2013) concluded that *"While PB produces liver tumors in rodents, important species differences were identified including a lack of cell proliferation in cultured human hepatocytes. The MOA for PB-induced rodent liver tumor formation was considered to be qualitatively not plausible for humans. This conclusion is supported by data from a number of epidemiological studies conducted in human populations chronically exposed to PB in which there is no clear evidence for increased liver tumor risk."*

Specific Comments Relating to MoA for Fluopyram-induced Rat Liver Tumours.

In a long-term study the non-genotoxic chemical fluopyram has increased the incidence of hepatocellular tumours in rats. Other studies have provided consistent, cohesive and compelling evidence that the MoA for fluopyram in rats is the activation of the xenosensing receptors CAR and PXR. The evidence is summarised as below:

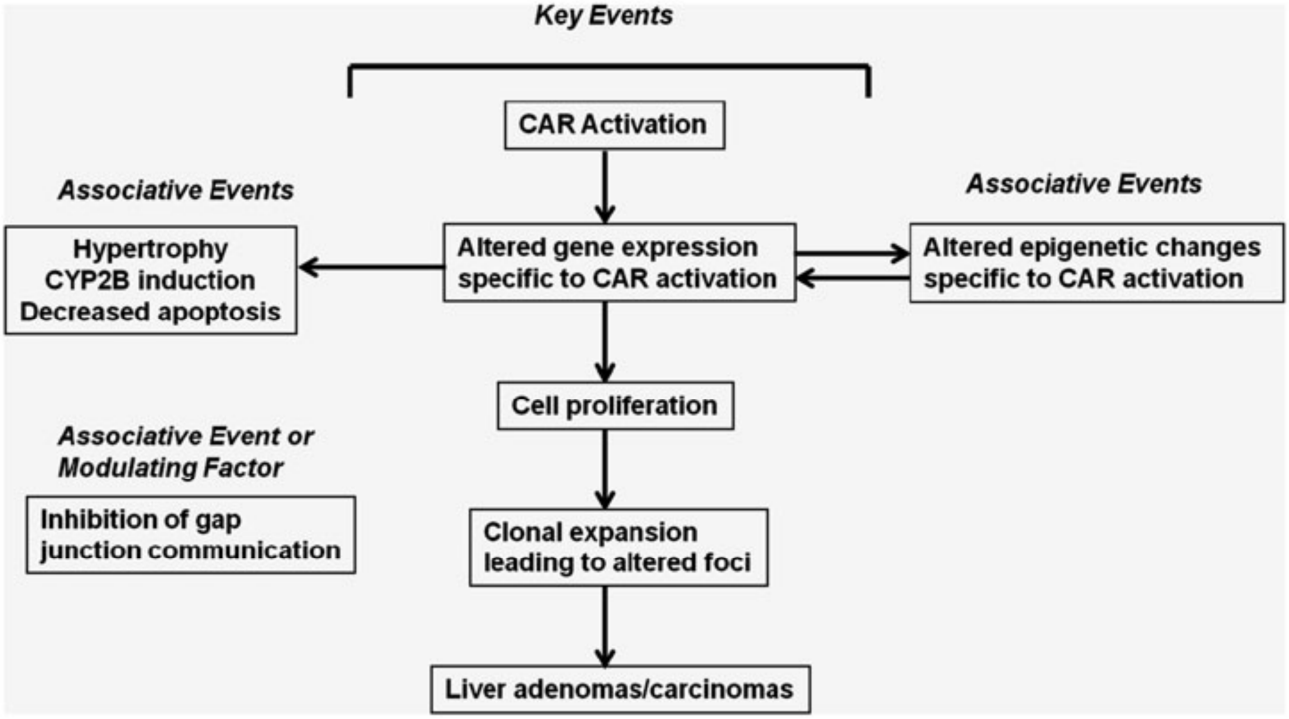
1. Hepatomegaly, centrilobular hypertrophy
2. Induction of CYP2B and CYP3A genes and associated enzyme activities
3. Stimulation of S-phase DNA synthesis in the absence of overt cytotoxicity
4. Observation of altered cell foci
5. Liver tumours
6. No liver enzyme induction nor microscopic changes in Car/Pxr knockout mice
7. Induction of CYP2B and CYP3A in rat and human hepatocyte cultures.
8. Stimulation of S-phase in cultured rat hepatocytes but **not** cultures of human hepatocytes.

These data unequivocally demonstrate a MoA involving a "phenobarbital-like" activation of CAR/PXR and the consequential species differences between rats and humans.

There is no consistent or convincing evidence to evoke a role for other potential MoAs (Cohen, 2010) for fluopyram. Other possible MoAs have been ruled out by:

1. The absence of induction of CYP4A by fluopyram demonstrates no involvement of PPARα.
2. No hypertrophic or hyperplastic effectsliver microscopic changes were seen in Car/Pxr knockout mice administered fluopyram.
3. No structural similarity of fluopyram with oestrogens.
4. No changes in clinical chemistry parameters & no hepatic focal necrosis or other histological changes suggestive of the involvement of other receptor- (e.g.oestrogen, statins) or non-receptor-mediated (e.g.cytotoxicity, infections, iron overload) mechanisms.

Figure 1. MoA for Phenobarbital-induced rodent liver tumour formation



In conclusion, the available data clearly identify fluopyram as a CAR/PXR-activator, stimulating hepatocellular cell proliferation as the key event in the MoA for hepatocarcinogenesis. In the absence of increased cell proliferation in human hepatocytes, this MoA is qualitatively **not** plausible in humans.

References.

Chamberlain M, Haines C, and Elcombe CR (2014). *Characterisation of the hepatic effects of phenobarbital in constitutive androstane receptor (CAR, NR1I3) knockout rats*. The Toxicologist In Press.

Cohen, S.M. (2010). *Evaluation of possible carcinogenic risk to humans based on liver tumors in rodent assays: The two-year bioassay is no longer necessary*. Toxicol. Pathol. **38**, 487-501, 2010.

Cohen SM, Meek ME, Klaunig JE, et al. (2003). *The human relevance of information on carcinogenic modes of action: overview.* Crit Rev Toxicol, **33**, 581–589.

Elcombe, C.R., Peffer, R.C., Wolf, D.C., et al., (2013). *Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator*. Crit. Rev. Toxicol. Early Online: 1–19, published on 17 October 2013.

Lake B. G. (2009). *Species differences in the hepatic effects of inducers of CYP2B and CYP4A subfamily forms: relationship to rodent liver tumour formation*. Xenobiotica, **39**, 582–596.

La Vecchia C and Negri E. (2013). *A review of epidemiological data on epilepsy, phenobarbital, and risk of liver cancer*. Eur J Cancer Prevent. doi:0.1097/CEJ.0b013e32836014c8

Ross J, Plummer SM, Scheer N, *et al*. (2010). *Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo.*
Toxicol Sci. **116**, 452–466.

Yamamoto Y, Moore R, Goldsworthy TL, *et al*. (2004). *The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice*. Cancer Res, **64**, 7197–7200.

Dossier Submitter's Response

The dossier submitter is grateful for the comment. However, the dossier submitter disagrees with this statement with respect to several issues:

a) It is postulated that there is no evidence for the involvement of other modes of action (MOA) in the fluopyram induced hepatocarcinogenesis than CAR/PXR activation. This hypothesis is, however, not supported by the mechanistic data presented. In the mechanistic studies provided by the applicant and summarised in the CLH dossier fluopyram has been shown to be a strong inducer of EROD activity in rats and

CYP1A1 expression in mice. These data are in contrast to findings on Phenobarbital (PB), which is not inducing CYP1A1 expression and only marginally increasing EROD activity. CYP1A1 expression and EROD activity are usually considered to be induced via other receptors than CAR/PXR. Hence it seems plausible to assume that other MOA are involved in the fluopyram mediated hepatocarcinogenesis.

b) It is stated that "a recent review and reanalysis of the available phenobarbital epidemiology suggests that it does not increase the incidence of liver tumours (La Vecchia and Negri, 2013)". However, in their review La Vecchia and Negri write that "… some, although not all, studies reported excess risk of all cancers and liver cancer in severe, but not in milder epileptics. There is no evidence of a specific role of phenobarbital in human liver cancer risk, but data on the topic are limited." The conclusion that epidemiological data are too limited to conclude whether or not Phenobarbital plays a specific role in human liver cancer risk would be a more appropriate summary of the data provided by La Veccihia and Negri. In this context it should be noted that PB itself is considered to be 'possibly carcinogenic to humans' by IARC.

Overall, the dossier submitter concludes that the classification and labelling of fluopyram with R40 / H351 is required.

RAC's response

Noted and discussion considered in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Germany	Bayer CropScience	Company-Manufacturer	4

Comment received

Response by Bayer CropScience (BCS) to Comment No. 1 *(ECHA note: Comment No. 8)* made by Member State Spain on Nov. 7, 2013, regarding uncertainties and outstanding data gaps pertaining to the mode of action (MoA) for the development of rat liver tumors following long term exposure to fluopyram and the relevance of these tumors to humans.

BCS believes that these concerns have now been fully addressed with the inclusion of new additional mechanistic data, as outlined in Comment No. 3 *(ECHA note: Comment No. 7)* submitted by BCS on Nov. 14, 2013.

The mechanistic data now clearly shows that the MoA by which fluopyram induces liver tumors in rats is specific and involves Car/Pxr nuclear receptor activation, as demonstrated by a 28-day mouse study using both the wild-type C57BL/6J mouse and a genetically modified mouse that does not have functional Car or Pxr receptors (Document No. M-449890-01-3). Activation of Car/Pxr receptors leads to hepatocellular proliferation, an essential precursory step for ultimate liver tumor formation by this MoA.

This vital step does not occur in humans as demonstrated in an in-vitro rat and human hepatocellular comparative assay (Document Nos. M-450157-01-2 and M-450156-01-2). Thus, the rat liver tumors resulting from exposure to fluopyram are not relevant to humans.

Detailed argumentation has now been submitted to convincingly exclude other plausible MoA's.

In light of the new mechanistic data, together with the lack of a genotoxic response in the standard battery of in-vitro and in-vivo genotoxicity studies conducted with fluopyram, additional investigative work to further rule out DNA reactivity as a possible MoA for the induction of liver tumors in rats is considered by BCS not to be warranted.
Dossier Submitter's Response
The dossier submitter thanks the notifier for the comment. However, some clarifications are needed: The new mechanistic studies provided do not sufficiently demonstrate non-relevance to humans of the liver tumor formation (see also more detailed responses to comment 3 and 7). With respect to the comment on genotoxicity we agree that no new studies are required, because the in vitro studies provided do not show any evidence of a genotoxic potential.
RAC's response
Noted and further discussed in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Norway		MemberState	5
Comment received				
The Norwegian Food Safety Authority considers the tumours induced by fluopyram in the liver of female rats and in the thyroid of male mice as relevant for humans. US EPA classified fluopyram as "Likely to be Carcinogenic to Humans". The Canadian PMRA came to the same conclusion. The phenobarbital mode of action proposed for the liver tumours is not clearly supported by the mechanistic studies conducted on female rats. The mechanistic studies conducted on mice to clarify the mode of action behind the thyroid tumours show a decrease in T4 and an increase in TSH. These changes are however not explained by the other mechanistic studies in which TPO and UDPGT activities were not affected. Organ specific genotoxicity and the formation of genotoxic metabolites cannot be excluded (see comment under mutagenicity).				
Dossier Submitter's Response				
During the joint global review classification of fluopyram as Carc. 1B / H350 "may cause cancer" has indeed been considered. However, since for the mode of action of thyroid tumors non-relevance to humans is assumed Carc. 2 / H351 "suspected of causing cancer" seems more appropriate (see also comment on new mechanistic studies on thyroid tumour formation below). The fact that TPO activity was not affected is not in conflict with the MOA postulated by the applicant. Inhibition of TPO would be a different mode of action of higher relevance to humans that had to be excluded as an alternative to the postulated MOA in accordance with the IPCS MOA framework.				
RAC's response				
Agree with DS response. For further details see the opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
14.11.2013	Germany	Bayer CropScience	Company-Manufacturer	6

Comment received

BCS agrees with the conclusion drawn on pages 149-152 of the CLH report that mechanistic data show that the thyroid tumors are not relevant to humans and should not be further considered for classification.

However to support fluopyram global registration and bring additional information on the MoA of fluopyram-induced thyroid tumors, additional mechanistic studies have been conducted by BCS in the mouse. These studies include
1) Fluopyram: 28-day dietary mouse studies (assess thyroid cell proliferation and reversibility) (Document Nos. M-428303-01-2 and M-449821-03-2)
2) Fluopyram: 28-day Wild Type versus Car/Pxr Knock Out mouse study (Document No. M-449890-01-3)
3) Fluopyram –Mechanistic investigations in the male mouse by oral gavage for 3 days (thyroid hormone investigations) (Document Nos. M-408352-01-2 and M-426994-01-2)
4) Fluopyram – Mechanistic investigation in the male mouse by dietary administration for 28 days (hepatotoxicity and thyroid hormone investigations) (Document No. M-428031-02-2)
5) Fluopyram – Definitive mechanistic 4-day toxicity study in the male mouse (pharmacokinetic investigations of the clearance of intravenously administered 125I-thyroxine) (Document No. M-328662-01-2)

As for the rat liver tumors, the 28-day Wild Type versus Car /Pxr Knock Out mouse study (Document No. M-449890-01-3), demonstrates that the initial trigger for the mouse thyroid tumors is Car/Pxr nuclear receptor activation in the liver. In this experiment, a significant induction of liver enzymes, liver enlargement, hepatocellular hypertrophy and thyroid follicular cell proliferation was seen in the WT mouse, but was not observed in the Pxr-Car KO mouse. The MoA for the thyroid tumors observed in the mouse includes the following key events: 1) Activation of the Car/Pxr receptors and increased activity of specific hepatic cytochrome P450 enzymes, associated with Car/Pxr activation, 2) Increased activity of specific uridine glucoronyltransferases (UGTs) associated with thyroxine (T_4) thyroid hormone clearance, 3) Increased levels of thyroid stimulating hormone (TSH), 4) Increased follicular cell proliferation, 5) Increased thyroid follicular cell hyperplasia that eventually proceeds to thyroid tumors.

Good dose and time concordance has been established for each key event, with the earlier events shown to be reversible.

Rodent thyroid tumors, which are secondary to induction of liver enzymes are widely accepted as being non-relevant for humans due to differences in thyroid physiology between rodents and humans (Capen, 1997; Dellarco et al 2006). 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.

In addition, as with the rat liver tumors, other plausible MoA's for thyroid adenomas in the

mouse have been effectively excluded.

BCS has submitted a concise synopsis of the mechanistic data generated to elucidate the MoA for rat liver tumors and mouse thyroid tumors and the relevance of these tumors to humans, which is presented in the following expert Position Paper "Fluopyram: Mode of Action and Human Relevance Analysis of Rodent Liver and Thyroid Tumors" (Document No. M-465168-01-1).

An in depth review, including detailed information on the mechanistic studies conducted, is presented in the following Expert Summary Report: "Fluopyram: Mode of Action and Human Relevance Framework Analysis for Fluopyram Induced Rodent Liver and Thyroid Tumors (Document No. 454439-01-1).

Since the limit for the file size to be uploaded on the ECHA homepage is 10 MB new data have been submitted on CD ROM to ECHA.

(*ECHA note: The following attachments were provided [Attachments 1 – 21]:*

1. Garcin, J. C., Wason, S., Van Goethem, D., Neumann, B., 2013, Tier 2 summary of the toxicological and toxicokinetic studies on the active substance for fluopyram (AE C656948) Extract Covering Mechanistic Data (Edition Number: M-470975-01-1)
[This document contains study summaries of the attachments below: Attachments 4-6, 9-11, 16-21]

2. Geter, D., Rouquie, D., Tinwell, H., Wason, S., 2013, Fluopyram: Mode of action and human relevance framework analysis for fluopyram-induced rodent liver and thyroid tumors (Edition Number: M-454439-02-1)

3. Wason, S., 2013, Fluopyram: Mode of action and human relevance analysis of rodent liver and thyroid tumors (Edition Number: M-465168-01-2)

ECHA note: The following attachments are full study reports and not published on the ECHA website:

4. 4. Confidential, 2012c, Fluopyram – 28 day toxicity study for proliferation assessment in the C57BL/6J male mouse (Edition Number: M-428303-01-2)

5. Confidential, 2013a, Fluopyram – 28 day toxicity study for thyroid cell proliferation in the C57BL/6J male mouse – Report amendment no 2 (Edition Number:M-449821-03-2)

6. Confidential, 2013b, 28-day dietary study to determine potential role of the nuclear pregnane X receptor (Pxr) and constitutive androstane receptor (Car) on the thyroid changes following the administration of fluopyram to male mice (C57BL/6J and Pxr KO/Car KO). (Edition Number: M-449890-01-3)

7. Capen, C. C., 1997, Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicologic Pathology, 1997. (Edition Number: M-435031-01-1)

8. Cohen, S. M., 2010, Evaluation of possible carcinogenic risk to humans based on liver tumors in rodent assays: The two-year bioassay is no longer necessary. Toxicologic Pathology, 2010. (Edition Number: M-367547-01-1)

9. Confidential, 2013e, Fluopyram: Assessment of pentoxyresorufin-0-depentylation and

benzyloxyquinoline-0-debenzylation in 50 liver microsomal samples (Edition Number: M-451628-01-1)

10. Confidential, 2013c, Fluopyram:-enzyme and dna-synthesis induction in cultured rat hepatocytes, main study hepatocytes, main study (Edition Number: M-450157-01-2).

11. Confidential, 2013d, Fluopyram: -enzyme and dna-synthesis induction in cultured human hepatocytes, main study hepatocytes, main study (Edition Number: M-450156-01-2)

12. Elcombe, C. R., Peffer, R.C., Wolf, D.C, Bailey, J., Bars, R., Bell, D., Catley, R.C., Ferguson, S. S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C. O., Schoeny, R., Xie, W., Lake, B. G., 2013, Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator, Crit Rev Toxicol. 2014 Jan;44(1):64-82. (Edition number: M-469431-01-1)

13. Confidential, 2013f, Tier 2 summary of the toxicological and toxicokinetic studies on the active substance for fluopyram (AE C656948) (Edition Number: M-301063-05-1)

14. Confidential, 2013g, Fluopyram: Mode of action and human relevance framework analysis for fluopyram-induced rodent liver and thyroid tumors (Edition Number: M-454439-01-1)

15. Hurley, P. M., Hill, R.N., Whiting, R. J., 1998, Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents, Environmental Health Perspectives, 1998. (Edition Number: M-086436-01-1)

16. Confidential, 2009, AE C656948 – Definitive mechanistic 4-day toxicity study in the male mouse (pharmacokinetic investigations of the clearance of intravenously administered 125I-thyroxine) (Edition Number: M-328662-01-2)

17. Confidential, 2011, Fluopyram – Mechanistic 3-day toxicity study in the mouse by oral gavage (thyroid hormone investigations) (Edition Number: M-408352-01-2)

18. Confidential, 2012a, Fluopyram – Mechanistic 3-day toxicity study in the mouse by oral gavage (thyroid hormone investigations) (Edition Number: M-426994-01-2)

19. Confidential, 2012b, Fluopyram – Mechanistic 28-day toxicity study in the mouse by dietary administration (hepatotoxicity and thyroid hormone investigations) (Edition number: M-428031-02-2)

20. Confidential, 2011b, Fluopyram – Mechanistic investigation in the female rat by dietary administration for up to 7 days (Edition Number: M-408029-01-2)

21. Confidential, 2012d, Fluopyram – Mechanistic investigation in the liver of female rats following dietary administration (Edition Number: M-427431-01-2)

Dossier Submitter's Response

The dossier submitter is grateful for the submission of further mechanistic data supporting the MOA for thyroid tumor formation and its quantitative non-relevance for humans. Thus classification and labelling with Carc 2 / H351 as proposed by the dossier submitter seems sufficient and classification with Carc 1B / H350 is not required.

RAC's response

The new studies have been considered by RAC while developing the opinion and are summarised in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
14.11.2013	Germany	Bayer CropScience	Company-Manufacturer	7

Comment received

Based on **new** mechanistic data generated, Bayer CropScience (BCS) strongly believes that the proposed classification and labelling of Carc. Cat. 3 (R40 - limited evidence of a carcinogenic effect) according to DSD criteria or Carc. 2 (H351 – suspected of causing cancer) according to CLH criteria, based on liver tumors observed in the rat, is no longer justified as the concerns raised on pages 149-152 of the CLH document, have now been fully addressed.

This proposed classification was based on the conclusion that uncertainties remained on the mode-of-action (MoA) data for rat liver tumors and other MoA's had not been convincingly ruled out, consequently, a conclusion on the non-relevance of the rat liver tumors to humans could not be made.

To address the concerns raised in the CLH report regarding rat liver tumors the mechanistic study programme now includes a 28-day Wild Type versus Car/Pxr Knock Out mouse study (Document No. M-449890-01-3), and comparative assessment of enzyme and DNA synthesis induction in cultured rat and human hepatocytes (Document Nos. M-450157-01-2 and M-450156-01-2). In addition, 7 and 28-day rat studies have been conducted at dose levels of 30, 75, 150, 600 and 1500 ppm fluopyram, i.e., dose levels used on the rat carcinogenicity study, plus intermediate dose levels, to assess multiple liver parameters (Document Nos. M-408029-01-2 and M-427431-01-2).

Taken as a whole, the mechanistic data clearly demonstrates that fluopyram induces rat liver tumors via a non-genotoxic MoA that involves the following key events: 1) activation of the constitutive androstane receptor (Car) and the pregnane X receptor (Pxr) in the liver and increased activity of hepatic cytochrome P450 (CYP) enzymes, specific for Car/Pxr activation, 2) Increased hepatocellular proliferation, 3) Increased incidence of altered hepatic foci that progress to liver tumors.

Good dose and time concordance has been established for each key event, with the earlier events shown to be reversible as demonstrated in the two additional repeat dose rat studies (Document Nos. M-408029-01-2 and M-427431-01-2). The MoA for fluopyram-induced rat liver tumors is similar to phenobarbital, a well-known Car-Pxr inducer.

A pivotal study to show Car/Pxr activation as the first key event was the 28-day mouse study using both the wild-type (WT) C57BL/6J mouse and a genetically modified mouse that does not have functional Car or Pxr receptors (Pxr-Car KO, Document No. M-449890-01-3). Both mouse strains were exposed to fluopyram at the tumorigenic dose (750 ppm) in the mouse cancer bioassay equivalent in terms of mg/kg/d to the rat tumorigenic dose level and above (1500 ppm). In this experiment, a significant induction of liver enzymes, liver enlargement and hepatocellular hypertrophy was seen in the WT mouse, but was not observed in the Pxr-Car KO mouse. This experiment clearly showed that mice lacking Car/Pxr receptors and exposed to fluopyram do not show the key event 1 which is necessary to initiate the tumorigenic pathway seen in the liver.

In order to demonstrate the non-relevance of rat liver tumors to humans, an in-vitro comparative study was conducted to examine the proliferative response in rat and human

primary hepatocytes exposed to fluopyram at a range of dose levels up to a cytotoxic dose (Document Nos. M-450157-01-2 and M-450156-01-2). In a similar manner to phenobarbital, used as a reference Car/Pxr inducer, the rat cells exposed to fluopyram showed a dose-response increase in proliferation, whereas human cells did not.
This *in-vitro* experiment clearly demonstrates that one of the crucial key events for progression of liver tumors (key event 2; hepatocellular proliferation), induced by fluopyram via Car/Pxr activation would not take place in humans.

The described MoA for rat liver tumors after long term exposure to fluopyram and the non relevance of these tumors to humans is supported by a recent publication (Elcombe et al, 2013, Crit Rev Toxicol, Early Online: 1–19, published on 17 October 2013), in which it was concluded that the MoA for phenobarbital-induced rodent liver tumor formation was qualitatively not plausible in humans.

Other plausible MoAs for liver tumor formation that are likely to be relevant to humans (Cohen, 2010) have effectively been excluded. The MoAs for liver tumorigenesis are broadly categorized as requiring increased cell proliferation through either receptor or non-receptor mediated processes or DNA reactivity (Cohen, 2010).

DNA reactivity is the first broad category of a MoA for hepatocellular carcinogens. The battery of regulatory guideline genotoxicity studies conducted on fluopyram shows that fluopyram does not have a genotoxic potential. Thus, DNA reactivity can be excluded as a potential MoA for the induction of tumors in rodents.

For non-DNA-reactive rodent liver carcinogens, several MoAs have been identified that act by stimulating hepatocellular proliferation through either a receptor or non-receptor-mediated mechanism. The MoA studies in rats and mice with fluopyram clearly demonstrate a specific, dose-related increase in the Cyp2b/Car associated and Cyp3a/Pxr-associated gene and enzyme activity. In addition, there was no increase of Cyp 4a1 (Pparα receptor), no structural similarity with estrogens, no changes in clinical chemistry parameters & no hepatic focal necrosis or other histological changes suggestive of other receptor (Estrogen, statins, and cytotoxicity) and non-receptor-mediated (cytotoxicity, infections, iron overload, and increased apoptosis) involvement. Furthermore, the absence of such a response in Pxr-Car-KO mice support the specificity for fluopyram-induced activation of Car and Pxr. An observed increase in Cyp1a1 gene transcript levels, a non-specific indicator of AhR activation, led to a minimal increase in ethoxyresorufin-O-deethylase (EROD) enzymatic activity (Document Nos. M 408029-01-2 and M-427431-01-2), indicating that activation of this receptor is most unlikely to be a key driver in promoting fluopyram induced liver tumors in the rat.

BCS has submitted a concise synopsis of the mechanistic data generated to elucidate the MoA for rat liver tumors and mouse thyroid tumors and the relevance of these tumors to humans, which is presented in the following expert Position Paper "Fluopyram: Mode of Action and Human Relevance Analysis of Rodent Liver and Thyroid Tumors" (Document No. M-465168-01-1).

An in depth review, including detailed information on the mechanistic studies conducted, is presented in the following Expert Summary Report: "Fluopyram: Mode of Action and Human Relevance Framework Analysis for Fluopyram Induced Rodent Liver and Thyroid Tumors (Document No. 454439-01-1).

Since the limit for the file size to be uploaded on the ECHA homepage is 10 MB new data have been submitted on CD ROM to ECHA.

In conclusion, BCS believes that based on extensive new mechanistic data generated, the proposed classification and labelling of Carc. Cat. 3 (R40) / Carc. 2 (H351) according to DSD and CLP criteria, respectively, based on liver tumors observed in the rat, is no longer justified as the concerns raised in the CLH report have now been fully addressed.

(ECHA note: The following attachments were provided: [Attachments 1 – 21])

1. Garcin, J. C., Wason, S., Van Goethem, D., Neumann, B., 2013, Tier 2 summary of the toxicological and toxicokinetic studies on the active substance for fluopyram (AE C656948) Extract Covering Mechanistic Data (Edition Number: M-470975-01-1)
[This document contains study summaries of the attachments below: Attachments 4-6, 9-11, 16-21]

2. Geter, D., Rouquie, D., Tinwell, H., Wason, S., 2013, Fluopyram: Mode of action and human relevance framework analysis for fluopyram-induced rodent liver and thyroid tumors (Edition Number: M-454439-02-1)

3. Wason, S., 2013, Fluopyram: Mode of action and human relevance analysis of rodent liver and thyroid tumors (Edition Number: M-465168-01-2)

(ECHA note: The following attachments are full study reports and not published on the ECHA website:)

4. 4. Confidential, 2012c, Fluopyram – 28 day toxicity study for proliferation assessment in the C57BL/6J male mouse (Edition Number: M-428303-01-2)

5. Confidential, 2013a, Fluopyram – 28 day toxicity study for thyroid cell proliferation in the C57BL/6J male mouse – Report amendment no 2 (Edition Number:M-449821-03-2)

6. Confidential, 2013b, 28-day dietary study to determine potential role of the nuclear pregnane X receptor (Pxr) and constitutive androstane receptor (Car) on the thyroid changes following the administration of fluopyram to male mice (C57BL/6J and Pxr KO/Car KO). (Edition Number: M-449890-01-3)

7. Capen, C. C., 1997, Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicologic Pathology, 1997. (Edition Number: M-435031-01-1)

8. Cohen, S. M., 2010, Evaluation of possible carcinogenic risk to humans based on liver tumors in rodent assays: The two-year bioassay is no longer necessary. Toxicologic Pathology, 2010. (Edition Number: M-367547-01-1)

9. Confidential, 2013e, Fluopyram: Assessment of pentoxyresorufin-0-dependentylation and benzyloxyquinoline-0-debenzylation in 50 liver microsomal samples (Edition Number: M-451628-01-1)

10. Confidential, 2013c, Fluopyram:-enzyme and dna-synthesis induction in cultured rat hepatocytes, main study hepatocytes, main study (Edition Number: M-450157-01-2).

11. Confidential, 2013d, Fluopyram: -enzyme and dna-synthesis induction in cultured human hepatocytes, main study hepatocytes, main study (Edition Number: M-450156-01-2)

12. Elcombe, C. R., Peffer, R.C., Wolf, D.C, Bailey, J., Bars, R., Bell, D., Catley, R.C.,

Ferguson, S. S., Geter, D., Goetz, A., Goodman, J.I., Hester, S., Jacobs, A., Omiecinski, C. O., Schoeny, R., Xie, W., Lake, B. G., 2013, Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator, Crit Rev Toxicol. 2014 Jan;44(1):64-82. (Edition number: M-469431-01-1)

13. Confidential, 2013f, Tier 2 summary of the toxicological and toxicokinetic studies on the active substance for fluopyram (AE C656948) (Edition Number: M-301063-05-1)

14. Confidential, 2013g, Fluopyram: Mode of action and human relevance framework analysis for fluopyram-induced rodent liver and thyroid tumors (Edition Number: M-454439-01-1)

15. Hurley, P. M., Hill, R.N., Whiting, R. J., 1998, Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents, Environmental Health Perspectives, 1998. (Edition Number: M-086436-01-1)

16. Confidential, 2009, AE C656948 – Definitive mechanistic 4-day toxicity study in the male mouse (pharmacokinetic investigations of the clearance of intravenously administered 125I-thyroxine) (Edition Number: M-328662-01-2)

17. Confidential, 2011, Fluopyram – Mechanistic 3-day toxicity study in the mouse by oral gavage (thyroid hormone investigations) (Edition Number: M-408352-01-2)

18. Confidential, 2012a, Fluopyram – Mechanistic 3-day toxicity study in the mouse by oral gavage (thyroid hormone investigations) (Edition Number: M-426994-01-2)

19. Confidential, 2012b, Fluopyram – Mechanistic 28-day toxicity study in the mouse by dietary administration (hepatotoxicity and thyroid hormone investigations) (Edition number: M-428031-02-2)

20. Confidential, 2011b, Fluopyram – Mechanistic investigation in the female rat by dietary administration for up to 7 days (Edition Number: M-408029-01-2)

21. Confidential, 2012d, Fluopyram – Mechanistic investigation in the liver of female rats following dietary administration (Edition Number: M-427431-01-2)

Dossier Submitter's Response

The mechanistic studies provided on the mode of action (MOA) for liver tumour formation show several weaknesses and conflicting data that taken together do not allow excluding human relevance.

a) As stated by several other comments there is evidence, that other MOA than the CAR/PXR dependent MOA are involved in fluopyram mediated liver toxicity. In the mechanistic studies provided by the applicant and summarised in the CLH dossier fluopyram has been shown to be an inducer of EROD activity in rats and CYP1A1 expression in mice. These data are in contrast to findings on Phenobarbital (PB), which is not inducing CYP1A1 expression and only marginally increasing EROD activity. CYP1A1 expression and EROD activity are usually considered to be induced via other receptors than CAR/PXR. Hence it seems plausible to assume that other MOA are involved in fluopyram mediated hepatocarcinogenesis.
b) The differences between PB and fluopyram are further substantiated by new studies on fluopyram (Ref 20). Besides the differences in CYP1A1 expression, which are

confirmed in female rats in a 7 day mechanistic analysis, there are other target genes induced by fluopyram, which are repressed by PB and vice versa. E.g. expression of Tacstd1 is significantly increased by fluopyram but repressed by PB.

c) In the new study with CAR/PXR KO mice provided by the notifier (Ref 6) there was still a significant increase in liver weight demonstrating that liver effects of fluopyram are not mediated by CAR / PXR alone. The findings on fluopyram are in contrast to published findings on PB in CAR-KO mice, where no such weight increase was observed (Yamamoto et al. Cancer Res 2004). These results further confirm, that fluopyram does not follow a classical 'PB-like' MOA but reveals its hepatotoxicity also by other MOA.
d) Additionally it should be noted that liver tumours were observed in female rats, and that most mechanistic studies have been performed in male mice. Thus these studies are considered of higher relevance with respect to the mechanism of thyroid tumour formation (which occurred in male mice). Furthermore – as criticised in the joint global review process – time concordance is missing since only 7 or 28 day studies have been provided.
e) In the study with primary human hepatocytes (Ref 11) cells of only one donor have been used. Since variability is known to be high among different donors and charges of primary hepatocytes the results of this experiment have to be critically questioned.

Overall, the dossier submitter concludes that the classification and labelling of fluopyram with R40 / H351 is required.

RAC's response

The new studies have been considered by RAC in developing the opinion and are summarised in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2013	Spain		MemberState	8

Comment received

The Spanish CA supports the proposed classification of Fluopyram as category 3 carcinogen, R40 based on Directive 67/548/EEC and as category 2 carcinogen; H351 based on Regulation EC/1272/2008.

There are two different types of tumours (liver and thyroid) in two different species (rat and mouse). For the thyroid tumours in male mice, mechanistic data suggest that they are not relevant to humans and they are not considered for classification. MoA supported: HPT disturbance by induction of T4 excretion, subsequent TSH increase, thyroid follicular cell hypertrophy and hyperplasia.

In female rats fluopyram was shown to have carcinogenic effects in the liver (adenomas and carcinomas). A number of mechanistic studies were performed to elucidate potential similarities between fluopyram and the well known tumour promoter phenobarbital (PB). PB is a chemical for which there is strong epidemiological data supporting non-carcinogenicity in humans. There is also significant evidence that increased cell proliferation observed in PB-induced liver tumours in rodents, does not occur in the human liver.

After administration of Fluopyram, findings consistent with a PB-like response are the induction of CYP450 of the 2B family, increased liver weight, hepatocellular hypertrophy, increased of liver cell proliferation and inhibition of apoptosis. The development of altered

hepatic foci is also a key event in the MOA for Phenobarbital-induced liver tumors. Like PB, the appearance of such foci, adenomas and carcinomas occurred only after chronic administration of Fluopyram. Other finding consistent with a phenobarbitone-like response are the lack of genotoxicity.

However, data for concordance analysis with PB are limited. There are a number of data gaps, such as the lack of available data regarding the constitutive androstane receptor (CAR) involvement in the induction of CYP2B isoforms following Fluopyram exposure. CAR dependency of PB-induced CYP2B induction was confirmed as PB does not produce liver tumours in CAR knockout mice. Although a CAR knockout rat has not to date been developed, the role of CAR in the CYP2B induction for Fluopyram has not been determinated using a recently developed RNA interference (RNAi) technique in CAR knockdown rat hepatocytes. Consequently, CAR dependency of this effect has not been confirmed.

It is also unclear why the administration of Fluopyram did not result in an enzyme induction profile in mice liver similar to that observed with Phenobarbital.

To define a MOA in liver, it is critical to ensure that other MOAs do not contribute significantly to hepatocarcinogenesis. It is important to ensure that DNA reactivity, other possible MOA for the induction of liver tumours in rats, is not the source of the tumour findings. In this sense, there is no data, such as DNA adducts analysis in liver cells, to assess whether hepatocelular tumours seen are attributable to specific mutagenic events.

Strength, consistency and specificity hepatic tumors in female rats of the suggested MoA is only partially convincing (e.g. gene expression analysis has shown that fluopyram partially induces other genes different from phenobarbital). Other MoA have not convincingly been excluded. CAR activation is not shown. Hence there are remaining uncertainties that do not allow concluding on non-relevance of these tumors to humans.

Therefore, based on current information for this compound, there is not robust data for a PB-like MOA. The results from the supplementary studies are not sufficient to eliminate the concern for the relevance of these tumours to humans. Relationships between Fluopyram activation pathways and their involvement in carcinogenesis should be further established. Given the uncertainties the classification regarding carcinogenicity can not be ruled out.

Overall, there is a clear carcinogenic effect in the liver of female rats (adenoma and carcinoma) of potential relevance to humans. Considering that only low incidences of liver carcinomas were observed in one specie and one sex was regarded of relevance, Fluopyram should be considered as carcinogenic category 3 according to DSD (R40)/ carcinogenic category 2 according to CLP (H351).

Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Norway		MemberState	9
Comment received				

The conducted genotoxicity studies show that fluopyram was negative for genotoxicity both in vitro and in vivo. Because of the tumorigenic effect of fluopyram, a second in vivo test to investigate organ specific genotoxicity should have been conducted.
Dossier Submitter's Response
It it is agreed that to fully investigate the mode of action for liver tumor formation an organ specific test addressing potential genotoxicity would have been helpful. Since none of the in vitro tests on genotoxicity was positive it is, however, believed that the data package provided is sufficient to exclude a genotoxic potential of fluopyram.
RAC's response
Noted.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Norway		MemberState	10
Comment received				
A classification in Repr. 2 H361d, according to CLP, is warranted based on the increased incidences of certain visceral and skeletal malformations/variations in rats and the malformation 'gall bladder absent' in rabbits.				
Dossier Submitter's Response				
The malformation 'gall bladder absent' occurring in rabbits in two fetuses at 75 mg/kg bw was not considered treatment related, because it was inside the historical control range. 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 and labelling for developmental toxicity. Hence, no classification of fluopyram with Repr. 2 H361d is considered necessary.				
RAC's response				
Reproductive toxicity was discussed by RAC and the outcome is reflected in the opinion.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
25.11.2013	Sweden		MemberState	11
Comment received				
The Swedish CA supports the environmental classification of Fluopyram (CAS 658066-35-4) as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard classes and differentiation. The lowest acute aquatic toxicity of fluopyram with ErC50 = 2.51 mg a.s./L(Lemna gibba, 7 d, stat.) is above the trigger for acute aquatic classification (1 mg/L). Therefore no acute aquatic classification is necessary. In the ELS study with Pimephales promelas the chronic aquatic toxicity of fluopyram was determined with a NOEC of 0.135 mg a.s./L. The study with aquatic plant Lemna gibba delivered a NOEC of 0.256 mg a.s./L. These values are higher than the trigger for chronic				

category 1 (NOEC ≤ 0.1 mg/L) but below the trigger for chronic category 2 (NOEC ≤ 1.0 mg/L). The results of the test on the biodegradation of Fluopyram in the water/sediment system and abiotic degradation show that Fluopyram is considered not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling. Fluopyram has a log Po/w of 3.3 (20°C). The experimentally derived steady state BCF of 13 L/Kg ww for fluopyram were obtained after lipid normalization to 5 % lipid content related to parent is below the trigger of 500 for not rapidly degradable substances. Taken together, these results warrant the environmental classification Aquatic Chronic Category 2 (H411). An M factor is not requested.
Dossier Submitter's Response
Thank you for your comments and agreement with environmental classification and labelling.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Germany	Bayer CropScience	Company-Manufacturer	12
Comment received				
CLH Report, Page 276, Table 246: The row Potential for or actual bioaccumulation / BCF = 62.5 / see Table 231 should be changed to Potential for or actual bioaccumulation / BCF = 13 / see Table 233 Justification: in Table 233 on page 267, Table 234 on page 268 and in the conclusion on page 277 the right value BCF = 13 is included.				
Dossier Submitter's Response				
Thank you for your comments. There is a typing error in table 246 on page 276 BCF = 62.5. The correct value is BCF = 13.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.11.2013	Belgium		MemberState	13
Comment received				
No acute toxicity was recorded in fish and invertebrates at levels close or up to the limit of the reported water solubility. Considering the aquatic acute toxicity for algae and aquatic plants , it can be concluded that Lemna gibba is the most sensitive species with a 7dEC50 of 2.6 mg/l (mm). Fish (Pimephales promelas) is the most sensitive species for aquatic chronic toxicity with a 33dNOEC of 0.135mg/l (TWA). Based on the results of the aquatic toxicity test on the most sensitive species , the fact that the substance is considered as not rapidly degradable it is justified to classify, following the classification criteria of regulation 1272/2008, as Aquatic chronic 2,H411. Furthermore, the substance shows low potential to bioaccumulate (BCF=13).				

Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, Fluopyram should be classified as N, R51/53.
SCL :
N, R50/53 : C≥25%
R52-53 : 2.5≤C<25%

In conclusion : we agree with the proposed environmental classification by the German dossier submitter.

Some editorial or/and minor comments
Not all tests on aquatic toxicity provided in the DAR were recorded in the CLH report.

Dossier Submitter's Response
Thank you for your comments and agreement with environmental classification and labelling
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.11.2013	France		MemberState	14

Comment received

FR agrees with the general conclusion dealing with the classification for environmental hazard of the substance. However, the NOEC value of 0.525 mg/L for *Chironomus riparius* seems to be based on TWA concentrations and not a mean measured concentrations. Then, the NOEC value of 1.39 mg/L, based on nominal concentrations, should be considered more relevant. The NOEC value of 1.39 mg/L is also the value validated in the EFSA journal 2013 of the substance. Moreover, could you, please, explain why the NOEC of *Lemna gibba* used in this CLH report is based on the $NOEC_{yield}$ instead of the $NOEC_{rate}$? These comments will not change the conclusion of the classification proposal.

Dossier Submitter's Response

Thank you for your comments and agreement with environmental classification and labelling.
For freshwater dipteran *Chironomus riparius* the NOEC value of 0.525 mg/L is based on time weighted average overlaying water concentrations (based on measurements of 53.6% at day 7 and 21.9% at day 28) for nominal test concentration of 1.39 mg/L. The nominal concentration of 1.39 mg/L is therefore the relevant NOEC value for this study.
For aquatic plant *Lemna gibba* the relevant NOEC value for classification purposes should be $NOEC_{rate}$ = 1.6 mg/L (nominal) instead of $NOEC_{yield}$ = 0.256 mg/L (nominal).

RAC's response

In the *Chironomus riparius* test (Dorgerloh, 2008) the measured concentrations, as reported in the DAR, are < 80% of nominal concentration during the test. Therefore the effect values should be related to mean measured concentrations, as stated in ECHA TG Chapter R.7b. In that case a NOEC of 0.525 mg ai/L should be preferred, based on TWA concentration that, as Time Weight Average, is the only mean measured concentration available.

For aquatic plant *Lemna gibba,* RAC agrees with Dossier Submitter's Response.

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
25.11.2013	France		MemberState	15
Comment received				
p7: It is given "data lacking" for most of physico chemical properties. As data are available to demonstrate that Fluopyram have no flammable, explosive or oxidising properties under DSD, could DE clarify that point. Even if some CLP specific requirement may be lacking, FR is of the opinion that, based on expert assessment, the "data lacking" should be changed to "conclusive but not sufficient for classification".				
Dossier Submitter's Response				
For some physico-chemical properties there is no need to collect date. Flammable gases, gases under pressure and further properties are not necessary, because "Fluopyram" is a solid. Data lacking could be appropriate for these points. For flammable solids, explosive or oxidising properties are data/statements available. For these properties the "conclusive but not sufficient for classification" is already in the CLH-report. The other points "data lacking" is sufficient.				
RAC's response				
Noted.				

ATTACHMENTS RECEIVED

1. Garcin, J. C., Wason, S., Van Goethem, D., Neumann, B., 2013, *Tier 2 summary of the toxicological and toxicokinetic studies on the active substance for fluopyram (AE C656948) Extract Covering Mechanistic Data* (Edition Number: M-470975-01-1) [*Filename: M-470975-01-1_M2_T2_Sect3_Point5_Toxicology Mechanistic Data_2013-12-02_Garcin_2013*] Submitted by Bayer CropScience on 14.11.2013 *[Please refer to comments 6 and 7]*
[This document contains study summaries of the attachments below: Attachments 4-6, 9-11, 16-21]

2. Geter, D., Rouquie, D., Tinwell, H., Wason, S., 2013, *Fluopyram: Mode of action and human relevance framework analysis for fluopyram-induced rodent liver and thyroid tumors* (Edition Number: M-454439-02-1) [*Filename: M-454439-02-1_IPCS Framework Document_Geter 2013*] Submitted by Bayer CropScience on 14.11.2013 *[Please refer to comments 6 and 7]*

3. Wason, S., 2013, *Fluopyram: Mode of action and human relevance analysis of rodent liver and thyroid tumors* (Edition Number: M-465168-01-2) [*Filename: M-465168-01-2_Fluopyram MoA position paper_Wason_2013*] Submitted by Bayer CropScience on 14.11.2013 *[Please refer to comments 6 and 7]*

FULL STUDY REPORTS (Not published on the ECHA website)

4. Confidential, 2012c, Fluopyram – 28 day toxicity study for proliferation assessment in the C57BL/6J male mouse (Edition Number: M-428303-01-2) *[Please refer to comments 6 and 7]*

5. Confidential, 2013a, Fluopyram – 28 day toxicity study for thyroid cell proliferation in the C57BL/6J male mouse – Report amendment no 2 (Edition Number:M-449821-03-2) *[Please refer to comments 6 and 7]*

6. Confidential, 2013b, 28-day dietary study to determine potential role of the nuclear pregnane X receptor (Pxr) and constitutive androstane receptor (Car) on the thyroid changes following the administration of fluopyram to male mice (C57BL/6J and Pxr KO/Car KO). (Edition Number: M-449890-01-3) *[Please refer to comments 6 and 7]*

7. Capen, C. C., 1997, Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicologic Pathology, 1997. (Edition Number: M-435031-01-1) *[Please refer to comments 6 and 7]*

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14. Confidential, 2013g, Fluopyram: Mode of action and human relevance framework analysis for fluopyram-induced rodent liver and thyroid tumors (Edition Number: M-454439-01-1) *[Please refer to comments 6 and 7]*

15. Hurley, P. M., Hill, R.N., Whiting, R. J., 1998, Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents, Environmental Health Perspectives, 1998. (Edition Number: M-086436-01-1) *[Please refer to comments 6 and 7]*

16. Confidential, 2009, AE C656948 – Definitive mechanistic 4-day toxicity study in the male mouse (pharmacokinetic investigations of the clearance of intravenously administered 125I-thyroxine) (Edition Number: M-328662-01-2) *[Please refer to comments 6 and 7]*

17. Confidential, 2011, Fluopyram – Mechanistic 3-day toxicity study in the mouse by oral gavage (thyroid hormone investigations) (Edition Number: M-408352-01-2) *[Please refer to comments 6 and 7]*

18. Confidential, 2012a, Fluopyram – Mechanistic 3-day toxicity study in the mouse by oral gavage (thyroid hormone investigations) (Edition Number: M-426994-01-2) *[Please refer to comments 6 and 7]*

19. Confidential, 2012b, Fluopyram – Mechanistic 28-day toxicity study in the mouse by dietary administration (hepatotoxicity and thyroid hormone investigations) (Edition number: M-428031-02-2) *[Please refer to comments 6 and 7]*

20. Confidential, 2011b, Fluopyram – Mechanistic investigation in the female rat by dietary administration for up to 7 days (Edition Number: M-408029-01-2) *[Please refer to comments 6 and 7]*

21. Confidential, 2012d, Fluopyram – Mechanistic investigation in the liver of female rats following dietary administration (Edition Number: M-427431-01-2) *[Please refer to comments 6 and 7]*