Committee for Risk Assessment

RAC

Annex 3

Response to comments document (RCOM)

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

[Reaction mass of 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*RS*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-*N*-[(1*RS*,4*SR*,9*SR*)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide [≥78% *syn* isomers ≤15% *anti* isomers relative content]; isopyrazam

EC Number: -
CAS Number: 881685-58-1

CLH-O-0000006915-65-01/F

Adopted

10 December 2020

ANNEX 3 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON REACTION MASS OF 3-(DIFLUOROMETHYL)-1-METHYL-N-[(1RS,4SR,9RS)-1,2,3,4-TETRAHYDRO-9-ISOPROPYL-1,4-METHANONAPHTHALEN-5-YL]PYRAZOLE-4-CARBOXAMIDE AND 3-(DIFLUOROMETHYL)-1-METHYL-N-[(1RS,4SR,9SR)-1,2,3,4-TETRAHYDRO-9-ISOPROPYL-1,4-METHANONAPHTHALEN-5-YL]PYRAZOLE-4-CARBOXAMIDE; ISOPYRAZAM

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: Reaction mass of 3-(difluoromethyl)-1-methyl-*N*-[(1RS,4SR,9RS)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide and 3-(difluoromethyl)-1-methyl-*N*-[(1RS,4SR,9SR)-1,2,3,4-tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide; isopyrazam
EC number: -
CAS number: 881685-58-1
Dossier submitter: United Kingdom (taken over by Norway)

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2019	Germany		MemberState	1
Comment received				
Regarding the solubility in organic solvents under section 7 of the CLH Report and in the document B.2, the solubility in dichloromethane is stated as 303 g/L. During our review, we found different values (303 g/L or 330 g/L) for the solubility in dichloromethane for the same reference. As the study report was not available, we could not check which value is correct. Please clarify.				
Dossier Submitter's Response				
Thank you for the comment. From a consideration of the study report, the correct value is 330 g/L and not 303 g/L as noted in the DAR and CLH report, apologies for the oversight.				
RAC's response				
RAC takes note of this comment and response.				

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Belgium		MemberState	2
Comment received				
BE CA would like to thank the UK Competent Authority for the submission of this CLH proposal. Overall, we support the conclusions proposed for all the physical and				

environmental hazard assessment, as well as STOT SE, skin corrosion and irritation, eye damage, skin sensitisation, and germ cells mutagenicity. However, we do have some comments regarding the carcinogenicity, reproductive toxicity and STOT RE endpoints.

Furthermore, we do have an identification issue with this substance. To guarantee a high level of health and environmental safety, BE CA is of the opinion that this CLH proposal should concerns isopyrazam as covered by the ISO name (which means a maximal content of 30% of the "anti" enantiomer) and not as primarily intended by the dossier submitter (e.g. a maximal content of 15% of the "anti" enantiomer); alternately if it is not conceivable, then we believe that two different entries (identification numbers) for each substance must be available in order to ensure a classification for all chemicals. In any case (1 or 2 separate entries), the full band of anti-enantiomers (0-30%) has to be covered by the harmonized classification.

Dossier Submitter's Response

Thank you for your comments.

With regards to the substance identification, in Commission Implementing Regulation (EU) No. 1037/2012 of 7th November 2012, the active substance is identified as follows:

Isopyrazam
CAS No 881685-58-1 (syn-isomer: 683777-13-1/ anti-isomer: 683777-14-2)
CIPAC No 963

The IUPAC names is given as:

A mixture of 3-(difluoromethyl)-1- methyl-N-[(1RS,4SR,9RS)-1,2,3,4- tetrahydro-9-isopropyl-1,4-methanonaphthalen-5-yl]pyrazole-4-carboxamide (syn-isomer – 50:50 mix of two enantiomers)
and
3-(difluoromethyl)-1-methyl-N- [(1RS,4SR,9SR)-1,2,3,4-tetrahydro-9- isopropyl-1,4-methanonaphthalen-5- yl]pyrazole-4-carboxamide (anti-isomer– 50:50 mix of two enantiomers)

In a range of 78:15% to 100:0% syn to anti.

The purity is given as:
≥ 920 g/kg in a range of 78:15% to 100:0% syn- to anti-isomers

As such, it is our understanding that the active substance identified as isopyrazam in the Implementing Regulation can contain a maximum of 15% of the anti isomer.

Further, from our discussions with the applicant, it is also our understanding that isopyrazam specifications have been developed to contain a maximum of 15% anti-isomer.

Consequently, the CLH report was proposed to apply to a maximal content of 15% anti isomer.

However, we welcome further consideration of this point to ensure consistency with the identification of the active substance (including the ISO name) and the material that is placed on the market.
RAC's response
Thank you very much. Noted. RAC supports the DS's view.

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	3
Comment received				
Syngenta supports the dossier submitter's conclusion on classification for carcinogenicity and disagrees with the dossier submitter's conclusion on classification for reproductive toxicity. Additional information related to hazard classes Carcinogenicity and Reproductive Toxicity is herewith provided. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20191121 CLH submission isopyrazam _Non-confidential.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20191121 CLH submission isopyrazam _Confidential.zip				
Dossier Submitter's Response				
Thank you for your comments and the attachments, these should be considered by RAC. The assessment of the DS is presented in the CLH report.				
RAC's response				
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2019	Germany		MemberState	4
Comment received				
The involvement of CAR in the mode of action was intensively studied in Annex 2: Mode of action and human health relevance assessment of the increased incidence of liver tumors in the female han wistar rat dosed with isopyrazam. The proposal uses an approach originally developed by IPCS/ILSI (2001). However, concerns about different modes of action for rodent liver tumor formation, e.g. AhR activation by complex HSP90-AhR release from AIP were not addressed in Table 3 of the document. The effects shown are apparently not limited to CAR activation but likely include activation of AhR related pathways. We further consider that there is evidence that for hepatocellular foci/adenoma there is a plausible alternative mode of action with relevance to humans. In addition, in regulatory accepted approaches such as AOP framework and for the possible use in risk assessment, the key event relationships (also used in Fig.1 of the document) have to be proven by weight of evidence analyses. The grades of low, moderate and strong were attributed to empirical support by a number and quality of studies also from open literature. Furthermore, inhibition studies demonstrating essential-ity for key events were not provided. Both empirical support and essentiality were not prov-en for isopyrazamin in the proposed MoA approach. Overall, the proposal of a mode-of-action hypothesis for				

induction of one possible pathway for liver tumours in rats does not exclude different MoAs as listed in the AOP Wiki database (AOP41: Sustained AhR activation leading to rodent liver tumours; AOP32: Inhibition of iNOS, hepatotoxicity, and regenerative proliferation leading to liver tumors, AOP46: AFB1: Mutagenic mode-of-action leading to hepatocellular carcinoma; etc.) and therefore does not exclude human relevance for these different MoAs as well. Thus, the classification as Carc. 2, H351 rather than non-classification appears more appropriate.
Dossier Submitter's Response
Thank you for your comments, these should be taken into consideration by RAC. The CLH report provides a consideration of other potential modes of action and the DS remains of the opinion that sufficient information is provided.
RAC's response
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	Switzerland	Federal Food Safety and Veterinary Office	National Authority	5
Comment received				
CH proposes to classify Isopyrazam as Carc. Cat. 2, in line with the argumentation for classification of Sedaxane as Carc. Cat. 2. Especially since the MoA analysis in the CLH report of Sedaxane is partly based on data with Isopyrazam. Isopyrazam and Sedaxane are structural analogs, and both induce treatment-related uterine adenocarcinomas at similar dose levels and comparable incidences. Human relevance of these tumors has to be very carefully analysed, especially in light of the ongoing discussion on a potential risk of SDHIs for humans (see e.g. discussions on Pydiflumetofen at the PRAS Meeting in September 2018). As long as the mode of action of SDHIs has not been fully understand elucidated, it is not possible to neglect the human relevance of uterine tumours found after treatment of rats with Isopyrazam and Sedaxane. SDHIs are supposed to lead to tumor formation by inducing epigenetic modifications through the accumulation of succinate (Letouzé et al. 2013), however, in the MoA Analysis for the uterine adenocarcinomas submitted by the applicant, epigenetic modifications were not considered as a potential mode of action for Isopyrazam induced tumours. Therefore, without an established MoA, which does not operate in humans, Isopyrazam should be classified as Carc. 2; H351.				
Dossier Submitter's Response				
Thank you for your comments, these should be taken into consideration by RAC along with the CLH report which presents the assessment of all relevant and available data on isopyrazam. We are aware of the RAC Opinion on sedaxane which referred to the additional data on isopyrazam. However, in the Opinion, it is noted that a robust assessment of the data on isopyrazam had not been presented at that time. The full data on isopyrazam should now be given due consideration. Regarding the comment that SDHIs are considered to lead to tumor formation by inducing epigenetic modifications through the accumulation of succinate (Letouzé et al. 2013), to				

the best of our knowledge, there is no convincing evidence that this occurs in mammals (including humans) in vivo. As pointed out by FR in comment 6 below, genetic defects of the SDH gene in humans lead to encephalopathies and cardiomyopathies, not to liver and uterine tumours. In addition, it should be pointed out that isopyrazam is extensively metabolised in vivo in the rat – therefore, it is unlikely SDH inhibition (with consequent succinate accumulation) does occur in vivo in mammals following exposure to isopyrazam.
RAC's response
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2019	France		MemberState	6
Comment received				
FR: Isopyrazam belongs to the chemical family of Succinate DeHydrogenase Inhibitors (SDHI), which rely on the inhibition of the fungal enzyme succinate dehydrogenase (mitochondrial complex II). As regards the potential mode of action underlying tumours formation, it is noteworthy that a high concern regarding the use of SDHIs as fungicides in agriculture has been raised by researchers and clinicians from French institutes with respect to the carcinogenic potential linked to SDH inhibition (Benit et al, 2018). This is based on human data where genetic mutations of SDH (leading to the loss of activity) are the cause of human diseases: - cell death (encephalopathies and cardiomyopathies) (Bourgeron et al. 1995 ; Parfait et al. 2000 ; Levitas et al. 2010) or - uncontrolled proliferation of cells causing cancer (Gimenez et al. 2002, 2003 ; Baysal et al. 2000 ; Burnichon et al. 2010 ; Janeway et al. 2010….). The tumour formation rather results from epigenetics modifications, which have been shown to be a long-term consequence of succinate accumulation, acting as an oncometabolite (Letouze et al. 2013). A report from an expert group set up by ANSES as well as the ANSES opinion published in January 2019 are available on line: https://www.anses.fr/fr/system/files/PHYTO2018SA0113Ra.pdf ANSES has informed EFSA, ECHA, DG Health and Food Safety and Competent Authorities about this raised concern. Increased incidences of hepatocellular and uterine tumours were observed in the rat carcinogenicity study with isopyrazam. Liver tumours: The proposed mode of action (MoA) for the increased incidence of liver tumours, involving the activation of CAR, could be considered plausible. Nevertheless, it is considered that uncertainties remain as some data are missing to substantiate this MoA (e.g. neither in vitro CAR/PXR assay nor data on gene expression were available, no CAR-Knock-Out animals were used…). Furthermore, it is noted that the data available to exclude the human relevance (if the postulated MoA would have been accepted) could be considered insufficient as only one donor was used in the study using human hepatocytes (in line with RAC opinion on Sedaxane (March 2019), a structurally related active substance of the same pesticide class (SDHI)).				

Uterine tumours:
The postulated MoA for the increased incidence of uterine carcinomas is not considered sufficiently substantiated by the available data, with high uncertainties regarding several key events.
It is noted that nearly the same MoA was proposed for sedaxane, a structurally related active substance of the same pesticide class (SDHI) showing the same type of tumours.
The 18-month carcinogenicity study conducted on isopyrazam was submitted during the harmonised classification and labelling process for sedaxane and considered by FR as Dossier Submitter during the commenting phase (see RAC opinion March 2019 and its Annex 2).
In addition to the general uncertainties related to the postulated MoA discussed by RAC in the context of sedaxane assessment, the specific uncertainties highlighted by FR for isopyrazam are the following:

* Key event three: Suppression of age-related decrease in dopaminergic signalling
- At the dose level of 3000 ppm, the mean dopamine concentrations in the median eminence of the hypothalamus were only statistically significantly higher at Week 26, and were not affected later (at week 52, week 66 and week 80).
- The measure of dopamine turnover in the median eminence was unaffected by treatment.
- Across the time points in this study, the concentration of dopamine and DOPAC in the median eminence remained fairly constant in the control animals from week 26 through week 80.
- According to the study report and Annex 3 of the CLH report (3.2.7), there was no difference in the amount of tyrosine hydroxylase staining in the arcuate nucleus by immunohistochemistry (for protein) or in situ hybridization (for RNA) between control and test substance-treated groups at week 52. There were also no test substance-related differences in the number of tyrosine hydroxylase-positive (dopaminergic) neurons in the arcuate nucleus between control and treated groups by unbiased stereology at weeks 66 and 80.
Therefore, these results do not support a decreased of dopamine with time (up to 80 weeks) in the control animals and a preservation of the dopaminergic activity with isopyrazam treatment, as postulated.

* Key events five and six: altered transition to reproductive senescence, increased total number of oestrus cycles and proliferation
- The 18-month isopyrazam study seems to suggest that the high dose level of 3000 ppm can delay the time of reproductive senescence onset. It is however noteworthy that in the GLP statement of the study report, it is mentioned that the systems used for calculation and tabulation of estrous cycle data were not validated.
- There were no apparent test substance-related effects (no dose response and/or no time-relationship) on proliferative lesions in the uterus, cervix and vagina in the 18-month isopyrazam study or in the 2-year rat study which is not in accordance with a proliferation process, as suggested.

In conclusion, FR is of the opinion that the experimental data do not provide enough evidence to support the postulated MoA of rat uterine tumours induced by isopyrazam. Furthermore, an alternative MoA through SDH inhibition and accumulation of succinate (considered as oncometabolite) could not be ruled out (see above).

As a conclusion, it is considered that classification of isopyrazam as Carc 2 is warranted based on uterine and liver tumours observed in rats.
Dossier Submitter's Response
Thank you for your comments, these should be taken into consideration by RAC along with the CLH report which presents the assessment of the DS. See also the response to comment number 5.
RAC's response
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Belgium		MemberState	7
Comment received				
In rats, in a 2-year chronic toxicity and carcinogenicity study (anonymous 2008a and anonymous 2009), an increase in liver adenoma (1.9, 0, 0, 5.8%in males and 0, 1.9, 1.9, 21.15% in females at 0, 100, 500 and 3000 ppm respectively) was observed. Contemporary HCD (2007-2009) in females show a rate of 0-1.9 % adenomas, but these HCD are extracted from only 3 studies and should be taken with caution. Also, an increase in the incidence of uterus carcinomas was reported with 1.9, 3.8, 5.8, and 28.8% of affected females at 0, 100, 500 and 3000 ppm (=0, 7, 35 and 233 mg/kg bw/d), respectively. The same remark as above is valid for the HCD (1.9-7.8 %). The incidence of carcinomas in relatively high considering the exposure levels. As only one study in available in rats, the consistency of this effect could unfortunately not be assessed. In mice, in an 80-week carcinogenicity study (anonymous 2008b), no neoplastic findings were reported. Nonetheless, BE CA would like to express its concerns regarding all the justification data provided by the dossier submitter. Could the induction of CYP be caused as an adaptative response of the liver, the target organ of Isopyrazam? Should we not maintain some reserves on the mode of action behind these carcinogenic effects? Are all uncertainties clarified?				
Dossier Submitter's Response				
Thank you for your comments, these should be taken into consideration by RAC along with the CLH report which presents the assessment of the DS.				
RAC's response				
Thank you very much. Noted. RAC will perform an in depth assessment of all uncertainties for the proposed modes of action. Nevertheless, RAC highlights that liver hypertrophy in response to enzyme induction is specifically considered as an adaptive response in the Guidance on the Application of the CLP Criteria				

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	8

Comment received

Agree with the dossier submitter's conclusion on classification for carcinogenicity.
The available data support the conclusion that isopyrazam does not pose a carcinogenic hazard to humans. Whilst administration of isopyrazam at dietary concentrations of 3000 ppm for 24 months to female Han Wistar rats resulted in higher incidences of hepatocellular adenomas and uterine adenocarcinoma, extensive MoA studies demonstrate that these are not relevant to humans. Sustained deficits in bodyweight gain were observed in females at 3000 ppm, which was approximately four-fold the recommended maximum tolerated dose (MTD).
The available data for isopyrazam support the proposed MoA and key events in rats and is well-described in the scientific literature. A sustained reduction in food utilization, reduced adipose tissue and leptin, affecting the hypothalamic feedback mechanisms, delaying age-related reductions in tuberoinfundibular dopaminergic (TIDA) neurons, which manifests as a reduction in prolactin, delaying reproductive senescence. The proportional increase in rats in persistent estrus lead to an increase in rats with elevated estrogen:progesterone ratios at the top dose, enhancing the proliferative stimulus to the uterine endometrium, resulting in an increase in spontaneous lesions in this tissue. Lower plasma levels of prolactin, led to reduced proliferative stimulus on the mammary and anterior pituitary glands, resulting in a reduction in tumour incidence. Thus, the shift in tumour incidence is dependent on a marked and sustained deficit in bodyweight gain, which was observed in female Han Wistar rats receiving 3000 ppm isopyrazam.
The applicant believes that the MoA assessment provides robust evidence of all key events, either directly or via associative events. Whilst steroid hormone levels were not specifically measured, oestrous cyclicity was considered a pragmatic and appropriate marker of the oestrogen:progesterone ratio given the significant technical and ethical challenges in female hormone measurement. Inherent intra- and inter- animal variability in estrogen and progesterone levels throughout the estrous cycle would have necessitated significantly larger groups, in order to sufficiently power the parameters. Furthermore, measurement of estrogen and progesterone would not have provided information on the key events regarding target tissues. No further data is considered necessary to support the MoA for uterine tumours.
Given the physiological control of the female reproductive cycle, and the drivers for reproductive senescence in humans are fundamentally different from those that occur in the rat, the MoA is not considered relevant to humans. Thus, the available data support the conclusion that the marginal increase in uterine tumours in isopyrazam treated rats does not pose a carcinogenic hazard to humans.
The available data for isopyrazam support a proposed MoA in female rats involving activation of the constitutive androstane receptor (CAR), leading to an early, transient, increase in hepatocellular proliferation and hepatocellular foci, which progress to form liver tumours. Contrary to rats, treatment of primary human hepatocytes (n=3) with isopyrazam had no effect on hepatocellular proliferation when tested up to the limit of cell viability. This pattern of effects matches the known species differences that have been demonstrated for other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MoA for isopyrazam between rats and humans. Numerous CAR knockout (KO) mice studies have been conducted to

demonstrate this MoA for model compounds, which has been successfully demonstrated via alternative in vitro methods. Consequently, no further data is considered ethically or scientifically justified to support the MoA for liver tumours. Thus, the available data demonstrates that this MoA is not relevant to humans and classification is not appropriate. Isopyrazam – Human Relevance Framework Assessment of Liver Tumour Induction in Female Rats attached to support the above statement. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20191121 CLH submission isopyrazam _Non-confidential.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20191121 CLH submission isopyrazam _Confidential.zip
Dossier Submitter's Response
Thank you for the comments and the attachments. These should be taken into consideration by RAC.
RAC's response
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2019	France		MemberState	9
Comment received				
FR : Page 60: - Delayed preputial separation and vaginal opening were considered by the DS to be secondary to reduced body weight gains and subsequent lower post-weaning body weights. Nevertheless, this statement should be substantiated by the available data (e.g. tabulated results including mean and range of the age and body weights at sexual maturation, historical control data…). - Decreased number of implantation sites and decreased mean litter size at birth were statistically significant at the high dose level in both generation. As only 2 studies are available for historical control data (HCD), it is more appropriate to consider concurrent control group rather than HCD. Page 76: The classification of isopyrazam Repr 1B H360D as proposed by the DS is supported, based on the consistent effects on the eyes observed in the offspring in 2 strains of rabbits in the 4 available studies.				
Dossier Submitter's Response				
Thank you for your comments and support for the proposed classification. Page 60 - As shown in table 43 of the CLH report, pup body-weight gain during lactation was reduced at the top-dose in both generations such that body weights were lower than controls at weaning. Further information relating to the preputial separation and vaginal opening is available from the study report as reproduced in the table below.				

	Dietary concentration (ppm)			
	0	100	500	3000
No of animals	26	26	26	26
Day of preputial separation (mean)	45.0	45.1	45.8	47.3**
SD	1.7	1.6	2.5	2.4
Mean body Weight at landmark (g)	171.9	170.4	171.0	159.2**
SD	15.7	14.5	19.5	17.6
Day of vaginal opening (mean)	36.2	36.9	36.6	38.2**
SD	1.2	1.8	1.8	2.0
Body Weight at landmark (g)				
Body Weight at landmark (g)	105.3	104.5	102.4	103.7
SD	7.8	11.2	8.4	13.8

With regards to the decreased number of implantation sites and decreased mean litter size at birth, it is noted that both were statistically significantly lower than the concurrent control values. However, whilst limited to two studies, it is the opinion of the DS that the available HCD do provide evidence to support the conclusion that these findings reflect normal background variation. These studies are relevant studies (in terms of the time they were undertaken and the laboratory) and should not be disregarded. They should be considered in a weight of evidence approach together with the other lines of evidence.

RAC's response

Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Belgium		MemberState	10

Comment received

Fertility
In an OECD 416 study performed on rats, at the highest dose of 3000 ppm, the total litter size was significantly decreased for both the F1 (-13%*) and F2 (-12%*) generations, in comparison with the controls; the number of implantation sites in the F0 and F1 mothers

were also significantly decreased (12.3 v.s. 10.7* and 12.8 v.s. 11.4*, in controls and F0 then F1 mothers, respectively) compared to controls. Concerning the historical control data (HCD), we acknowledge they are contemporary to the actual study, but we would like to highlight the fact that only a very limited number of studies was used to create these HCD (only 2) and therefore their relevance might be discussed. Considering these effects appear at a relatively low dose (close to 300 mg/kg bw/d), in two generations, we are of the opinion that these effects matter for classification of fertility and would like the dossier submitter to consider a Repr. 2; H361 classification for this endpoint.

Developmental toxicity
In an OCED 414 in rats (Isopyrazam 93:7), at 250 mg/kg bw/d, an increase in post-implantation loss and in the incidence of incomplete ossification of several foetal bones (cervical centra (5.6 – 35.7%**), sternum (12.4%**), caudal arches (4 – 8%), hind-paw bones (17%**) and fore-paw bones (8%**)) was reported as well as a decrease in mean foetal body weight. This was correlated with maternal effects such as a decrease in body weight gain.

In another OECD 414 study in rats (Isopyrazam 70:30), at 200 mg/kg bw/d, a decrease in mean foetal body weight and an increase in foetal visceral variations (50% v.s. 35% in controls) as well as in the incidence of non-ossification in several bones (vertebral centra and hind limbs phalanges) were reported. In mothers, at the same dose level, body weight gain was reduced and sedation was observed in all females when the study started. At 75 mg/kg bw/d, while body weight gain was reduced in mothers, the foetal mean body weight was also decreased (-6 % **, in comparison with the controls) and non-ossification was observed in one vertebral centrum.

In rabbits, three dose-range finding studies and one OECD 414 are available. Consistency was noted between the studies with reduced size of the eye (observed in Anonymous 2008b at 400 mg/kg bw/d without any maternal toxicity as "slightly reduced eye size", in Anonymous 2008c at 600, 800 and 1000 mg/kg bw/d without any maternal toxicity as "small eyes (malformations)" or "slightly small eye (variations)" or microphtalmia, in Anonymous 2008a at 700 and 1000 mg/kg bw/d as "eye malformation" but in presence of maternal toxicity such as decreased BW-gain and FC, decreased faeces production, increased GGT, increased relative liver weight, hepatocellular hypertrophy, and centrilobular hepatocellular vacuolation; and finally in Anonymous 2008b at 500 mg/kg bw/d as microphtalmia in presence of maternal toxicity noted as decreased faeces production, decreased FC, increased absolute liver weight, hepatocellular hypertrophy, and centrilobular hepatocellular vacuolation).

On the basis of the severe adverse effects seen not only in the rabbit (microphtalmia) but also in the rat (non-ossification) plus, not necessarily reported in presence of maternal toxicity, at doses relatively low (starting at 75 mg/kg bw/d in the rat and at 400 mg/kg bw/d in the rabbit), BE CA supports the proposal to classify Isopyrazam as Repr. 1B; H360.

In conclusion, we would be in favour of a Repr. 1B; H360Df for Isopyrazam.

Dossier Submitter's Response

Thank you for your comments, these should be taken into consideration by RAC.

It is noted that the mean litter size at birth was statistically significantly lower than the concurrent control values in the F1 and F2 generations and the number of implantation sites in the F0 and F1 mothers were also significantly decreased. However, we remain of the opinion that the available HCD (whilst limited to 2 studies) do provide evidence to support the conclusion that these findings reflect normal background variation. As such, we do not consider that these findings provide sufficient evidence to support classification of the substance. See response to comment 9 also. A consideration of the post-implantation loss and effects on ossification in the rat are considered in the CLH report.
RAC's response
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC. However, RAC supports the DS's view on these issues.

Date	Country	Organisation	Type of Organisation	Comment number
21.11.2019	Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	11
Comment received				
Disagree with the dossier submitter's conclusion on classification for reproductive toxicity. There is no evidence that isopyrazam causes adverse effects on sexual function, fertility or development in the rat. The only finding of relevance to classification for reproductive toxicity is the observation of microphthalmia and small eyes at high dose levels in the rabbit (≥600 mg/kg/day). Microphthalmia was only observed in the presence of marked maternal toxicity and is considered likely to be due to a non-specific secondary mechanism of disturbed homeostasis. Rabbit studies on isopyrazam were performed by two separate contract research organizations (CRO). Outsourced during the closure of the Central Toxicology Laboratory (CTL, Alderley Park, UK), the isopyrazam rabbit prenatal developmental toxicity studies were some of the first externally managed studies conducted for Syngenta. At RCC (Füllinsdorf, Switzerland), two preliminary studies in Himalayan rabbit reported foetal findings of small eyes, which were not sufficient to be described as microphthalmia. Small eyes had not previously been described by RCC and were not in the laboratory's glossary of foetal effects. Consequently, there were uncertainties in the procedures in place for minimising bias and reporting at RCC. Histopathological assessment of coronal head sections from 80 of the 115 foetuses examined macroscopically was conducted to verify RCC's reporting of "small eyes". The experimental design of the histopathological assessment reflected the primary purpose of the study – to clarify the findings of "small eye". The number of foetuses examined was not comparable across groups, skewing the incidence data, and the assessment was not blinded, nor evaluated by litter. Whilst the conclusions of Cartwright & Wright (2008) support the foetal observations, it is important to note that they do not supersede them. Syngenta changed CRO during isopyrazam development, which necessitated changing rabbit strain – due to limited breeders and users of the Himalayan rabbit. Preliminary and definitive prenatal developmental toxicity studies were conducted at WIL Research Laboratories (Ashland, US) in New Zealand White rabbits. In the preliminary study in New Zealand Whites, a higher incidence of microphthalmia was noted at 1000 mg/kg/day, which was considered related to administration of Isopyrazam. However, the maternal toxicity at this dose level was excessive (severe weight loss and abortion necessitating termination), and a top dose of 500 mg/kg/day was selected for the definitive				

developmental toxicity study. Increased liver weight and centrilobular hepatocyte hypertrophy was noted in New Zealand White rabbits from 150 mg/kg/day. In the definitive regulatory rabbit study, there were no occurrences of malformations outside of the laboratories historical control data range. The applicant believes that microphthalmia / small eyes in one species at dose levels exhibiting excessive toxicity and marked perturbations in liver function, can only constitute some evidence. The evidence is not sufficiently clear for Category 1b 'Presumed human reproductive toxicant' (H360D). The lack of any evidence of a treatment related increase in major malformations in the definitive regulatory studies should be considered the most significant factor in the judgement that there is insufficient evidence to support classification in Category 1b. Category 2 'suspected of damaging the unborn child' (H361d) is considered the only suitable remaining category. Technical Position on the Classification of Isopyrazam for Developmental Toxicity in Rabbits attached to support above statement. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 20191121 CLH submission isopyrazam _Non-confidential.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment 20191121 CLH submission isopyrazam _Confidential.zip
Dossier Submitter's Response
Thank you for your comments and the attached documents. These should be taken into consideration by RAC. However, the DS remains of the opinion that classification for Repr 1B (H360D) is appropriate.
RAC's response
Thank you very much. Noted. Your comments will be presented in the opinion and discussed by RAC.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2019	France		MemberState	12
Comment received				
FR: Acute oral toxicity: No data are available on isopyrazam containing 15% of the anti isomer. Acute oral toxicity studies showed LD50 > 2000 mg/kg bw with a batch containing 93:7 syn:anti isomers and LD50<2000 mg/kg bw with a batch containing 70:30 syn:anti isomers. Therefore, it cannot be excluded that the LD50 would be less than 2000 mg/kg bw with isopyrazam containing 15% of the anti isomer and a classification Acute Tox 4 is proposed.				
Dossier Submitter's Response				
Thank you for your comments and we welcome discussion of this point. There are no available data on isopyrazam containing ≤ 15% of the anti isomer that support classification for acute oral toxicity. The available LD_{50} values for the pure isomers and various isomer ratios are as follows: Pure Syn > 2000 mg/kg bw 93:7 syn:anti > 2000 mg/kg bw				

70:30 syn:anti < 2000 mg/kg bw 50:50 syn:anti = 310.2 mg/kg bw Pure Anti = 310.2 mg/kg bw Considering the ATE of the pure anti isomer, the ATE of the substance containing ≤ 15% could be calculated to be > 2000 mg/kg bw.
RAC's response
Thank you very much. Noted. RAC supports the DS's view.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Belgium		MemberState	13
Comment received				
Concerning the oral route, BE CA is of the opinion that the up-and-down procedure (OECD 425; anonymous 2008a) performed on rats with isopyrazam (syn:anti ratio 70:30) should be considered with more attention and not be taken as additional information. We note that this CLH proposal is intended for isopyrazam containing a maximum of 15% of the anti enantiomer; but as the provisional ISO name correlated with this substance can contain up to 30% of the anti enantiomer, a classification as acute tox. 4; H302 should be considered.				
Dossier Submitter's Response				
Thank you for your comments. Please see the response to comment number 2 regarding the identification of the substance. As noted in the response to comment 12, there are no data on the substance containing ≤ 15% of the anti isomer that would support classification for acute oral toxicity. However, we agree that the data on the substance with a 70:30 syn:anti isomer ratio would meet the criteria for classification as Acute Tox 4; H302. We would welcome further consideration of the classification.				
RAC's response				
Thank you very much. Noted. RAC notes that the CLH-report is released for preprations containing up to 15% of anti isomer.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Belgium		MemberState	14
Comment received				
BE CA acknowledges that the liver is the target organ of Isopyrazam, as this organ is consistently affected across the studies (mostly statistically significant increased relative liver weight, hepatocellular hypertrophy, increased hepatic enzymes and cholesterol…), and in different species (rat, mouse, rabbit, dog…). However, or most of adverse the effects appear at doses not relevant for classification or the effects seen at doses relevant for classification are not considered as severe enough				

to trigger a classification as STOT RE.
Dossier Submitter's Response
Thank you for your comments. We agree that the available data do not support classification for STOT RE.
RAC's response
Thank you very much. Noted.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
12.11.2019	Germany		MemberState	15
Comment received				
We agree with the proposal of classification for environmental hazards as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 and the acute/chronic M-factor of 10. Supplemental information is available for the degradation product "metabolite CSCD465008". There is another valid test for freshwater algal growth inhibition according to OECD Guideline 201 with Pseudokirchneriella subcapitata SAG.61.81 (Zmijowski, G., 2009). Relevant endpoints are EyC50 (72 hours) = 22.44 mg/L, ErC50 (72 hours) = 26.52 mg/L and NOEC (72 hours) -= 18 mg/L. These data are not relevant for classification and labelling of isopyrazam itself, but relevant for submitted Annex IV - Ecotoxicity degradant information in the active substance approval process.				
Dossier Submitter's Response				
Thank you for your comments supporting the classification proposal of isopyrazam. We note the comments regarding the metabolite.				
RAC's response				
RAC takes note of this comment and response. RAC takes into account the note on the available data showing the degradation products are not considered more toxic than the parent substance and agrees with the DS to not take into account the information for classification purposes.				

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2019	Sweden		MemberState	16
Comment received				
p. 93-114; Evaluation of environmental hazard: The Swedish CA agrees with the proposed environmental classification; Aquatic Acute 1, H400 (Acute M-factor = 10) and Aquatic Chronic 1, H410 (Chronic M-factor = 10). This classification proposal is based on several studies evaluated earlier during the process under Directive 91/414/EEC considering evaluation of active substances of plant protection products. Technical isopyrazam contains a mixture of two diasteroisomers designated syn and anti-isomers. Both of the isomers are considered to be biologically active. However, toxicity testing with fish indicates that the anti-isomer may be more ecotoxic than the syn-isomer. The Swedish CA therefore agrees to exclude the study on the anti-isomer and only include studies with a representative mixture of the two isomers as has been done in the proposal.				

Dossier Submitter's Response
Thank you for your comments supporting the classification proposal.
RAC's response
RAC takes note of this comment and response. RAC notes the composition of isopyrazam and agrees with the DS to base the classification of isopyrazam on the available test data using 70:30 isomer ratio. RAC agrees with the DS that based on the available data the anti-isomer appears to be more acutely toxic to fish and invertebrates than the syn-isomer.

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2019	France		MemberState	17
Comment received				
FR agrees with the proposed classification and M factors.				
Dossier Submitter's Response				
Thank you for your comments supporting the classification proposal.				
RAC's response				
RAC takes note of this comment and response.				

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2019	Belgium		MemberState	18
Comment received				
BE CA supports the proposed environmental classification based on the data in the CLH dossier: Aquatic Acute 1, H400 ; M=10 Aquatic Chronic 1, H410 ; M=10 A clear difference in toxicity in fish was observed between the syn and anti-isomer, with higher toxicity observed for the anti-isomer. This is also reflected in the outcomes of the 90:10 versus 70:30 syn:anti isomer ratios where the latter showed higher toxicity (1 order of magnitude). The same trend is also observed in the Daphnia magna studies performed with both ratios. Unfortunately, studies with algae were only performed with 90:10 syn:anti ratio and results might does not reflect the true toxicity. Some editorial or/and minor comments : Table 67: for the study with Lemna gibba a static study regime is mentioned, while a semi-static is mentioned in the description of the study underneath				
Dossier Submitter's Response				
Thank you for your comments supporting the classification proposal. We agree that the *anti* isomer appears to be more acutely toxic to fish and invertebrates than the *syn* isomer with an approximate order of magnitude between the endpoints for the 90:10 and 70:30 *syn:anti* ratios. The position is unclear with regard to chronic endpoints as only the 70:30 *syn*:*anti* ratio was tested.				

The available algal endpoints were determined with the 70:30 *syn*:*anti* ratio which is within the broader technical specification for isopyrazam. If the order of magnitude observed with fish / invertebrates is applied to the available algal endpoints determined with the 70:30 ratio, we anticipate endpoints with a 90:10 ratio would not impact the classification as estimated endpoints are not lower than acute and chronic fish endpoints. We confirm the Everett *et al*, 2007 *Lemna* study employed a semi-static regime
RAC's response
RAC takes note of this comment and response. RAC notes the composition of isopyrazam and agrees with the DS to base the classification of isopyrazam on the available test data using 70:30 isomer ratio. RAC agrees with the DS that based on the available data the anti-isomer appears to be more acutely toxic to fish and invertebrates than the syn-isomer. As for the chronic toxicity test data is available only for 70:30 syn:anti ratio it is not possible to conclude on the chronic toxicity of the individual isomers. RAC agrees with the DS on the algae toxicity that the anticipated endpoints with a 90:10 ratio are not lower than acute and chronic fish endpoints and would not impact the classification.

PUBLIC ATTACHMENTS
1. 20191121 CLH submission isopyrazam _Non-confidential.zip [Please refer to comment No. 3, 8, 11]

CONFIDENTIAL ATTACHMENTS
1. 20191121 CLH submission isopyrazam _Confidential.zip [Please refer to comment No. 3, 8, 11]