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 the comments displayed below may have been accompanied by attachments which are not published in this table.

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Last data extracted on 01.08.2019

Substance name: N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide; isoflucypram
CAS number: -
EC number: -
Dossier submitter: United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
24.07.2019	Germany		MemberState	1
Comment received				
The German CA agrees with the classification of isoflucypram as proposed by the UK CA.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
24.07.2019	Germany	Bayer AG	Company-Manufacturer	2
Comment received				
With regard to carcinogenicity (10.9) section, we (Bayer) generally agree with the assessment. In addition to the data presented in the carcinogenicty (10.9) section of the CLH report, we would like to submit an in vitro characterisation of isoflucypram and the metabolites identified in plasma samples during the 2 year rat carcinogenicity study (SA 13266) (The full report (M-665264-01-1) is attached to this comment). In the standard rat 28 day (SA 11308) and 90 day (SA 12102) studies, both the liver and thyroid were identified as target organs of isoflucypram. In addition, mechanistic studies had determined that the effects observed in these two organs were initiated by CAR/PXR activation. However, contrary to expectations, no adverse effects were observed in the liver or thyroid at the end of the rat 2-year carcinogenicity study. The absence of any appreciable amounts of isoflucypram in plasma samples taken at various time points during the cancer bioassay, coupled with significant levels of two metabolites in the same samples led to the conduct of preliminary in vitro mechanistic screens. A third metabolite, detected in the 24-month samples, was also included in the investigations. The objective of these preliminary in vitro screens was to understand the disparity between the expected and the actual outcome in the long-term rat study. Consequently, they were designed to determine the potential of each of the identified plasma metabolites, as well as isoflucypram, to activate CAR/PXR and to induce expression of genes specific to these nuclear receptors. The in vitro screens confirmed that isoflucypram is a CAR/PXR agonist and, thus, has the				

potential to induce CAR/PXR mediated liver effects and liver-mediated thyroid effects (see M-665264-01-1, Figure 4 & 5). In contrast, the in vitro data indicated that, under the same conditions of test, the metabolites are not CAR/PXR agonists and do not have the potential to induce CAR/PXR mediated liver and liver-mediated thyroid effects (see M-665264-01-1, Figure 4 & 5).

The differences in response between isoflucypram and its major plasma metabolites may help to explain the unexpected lack of liver and thyroid findings in the long-term rat study.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment M-665264-01-1.pdf

Date	Country	Organisation	Type of Organisation	Comment number
24.07.2019	Germany	Bayer AG	Company-Manufacturer	3

Comment received

With regard to the carcinogenicity (10.9) section, we (Bayer) generally agree with the assessment.

In addition to the data presented in the carcinogenicity (10.9) section of the CLH report, we would like to submit a physiologically-based toxicokinetic (PBTK) modeling study of isoflucypram in rat (The full report is attached to this comment). The scope of this PBTK modeling study was to assess the isoflucypram plasma exposure that could be expected in rats by means of a PBTK modeling approach. It is well established that exposure to a chemical substance and the bioavailable amount of the dose are often not identical [Creton et al., 2009]. Describing the toxico-kinetic profile of isoflucypram using PBTK modeling allows extrapolating between doses and administration routes. With this, the internal systemic plasma exposure can be assessed as a function of dose. PBTK modeling allows the transfer from a dose-response to an exposure-effect relationship, in terms of internal exposure.

The results of the PBTK modeling indicate that, in the rat, the plasma exposure of isoflucypram saturates around a dose of 30 and 40 mg/kg/day for female and male rats respectively (see M-665315-01-1, figure 16; document attached).

The predicted exposure saturation at about 30 and 40 mg/kg/day for female and male rats correlate well with the top dosage used for the 2 year rat carcinogenicity study (SA 13266). The female top dose is on average 46.6 mg/kg/day and, thus, exceeded the kinetically-derived dose by 1.5-fold. The male top dose (~18.6 mg/kg/day) of the 2-year study is roughly 2-fold lower than the predicted dose based on the PBTK model. From the purely toxicokinetics perspective, a higher dose for male rats would have been reasonable. However, a discrepancy of a factor two seems to be manageable if this is correctly taken into account in the risk assessment, e.g., in terms of reference dose or chemical-specific adjustment factor.

References:
[Creton et al., 2009] Creton S, Billington R, Davies W, Dent MP, Hawksworth GM, Parry S, and Travis KZ (2009) Application of toxicokinetics to improve chemical risk assessment: implications for the use of animals. Regul Toxicol Pharmacol 55(3):291-299. doi:10.1016/j.yrtph.2009.08.001.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment M-665315-01-1.pdf

Date	Country	Organisation	Type of Organisation	Comment number
24.07.2019	Germany	Quality Scientific Solutions	Please select organisation type..	4

Comment received

ECHA has invited the public to comment on the hazard classes for isoflucypram (1). The basis for hazard classification and labeling has been summarized by the UK Competent Authority (2). In the section labeled "Deficiencies" in the UK review of the chronic toxicity/carcinogenicity study in the rat and the two-generation reproduction study, the reviewer indicated that the studies complied with the technical relevant guidelines although no adverse findings were noted. At the request of Bayer CropScience, Quality Scientific Solutions, determined if the maximum tolerated dose (MTD) was reached or exceeded in the multigeneration study and the sub-chronic, chronic and lifetime studies in mouse, rat, and dog on the succinate dehydrogenase inhibiting (SDHI) fungicide, isoflucypram (FRAC Code C2 (3)). The hazard identification profile for isoflucypram was examined, and its absorption, distribution, metabolism, and elimination were characterized. Based on this assessment, body weight gain reduction was selected as the primary toxicological indicator of toxicity (4).

Dose-response relationships observed for isoflucypram were compared to dose-response relationships observed for five other pyrazole carboxamide fungicides (bixafen, fluxapyroxad, penflufen, sedaxane, and solatenol) for which there was publicly available data. Benchmark doses (BMDs) were calculated on body weight gain data, a measurement common to all SDHI fungicides. U.S. EPA's Benchmark Dose Software (BMDS, Version 2.6.0.1) was used to fit the Hill model (with summarized means and standard deviations) for "body weight gain (% of control)" versus dose (mmole/kg body weight/day). The Benchmark Response (BMR), which was defined as the point (dose) corresponding to a 10% decrease in body weight gain relative to that of the control group, was calculated when the dose-response could be fitted by the Hill model. BMDs were calculated for isoflucypram based on data from sub-chronic and chronic studies conducted in the rat and the chronic mouse and dog studies and the rat reproduction study. BMDs were calculated on body weight gain data from chronic rat studies reported for each of the five pyrazole carboxamide fungicides to compare the magnitude of these SDHI fungicides to isoflucypram. Average daily doses (mg/kg/day) administered during each interval for which body weight gain was calculated, were converted to a common dose scale (millimole/kg body weight/day) based upon the SDHI's molecular weight. For each SDHI for which there was data, the mmole/kg/day doses were plotted vs. body weight gain to visualize and compare the effect of the SDHI fungicides on body weight gain (Figure 1). As an alternative to BMD analyses, linear regression modeling was used to quantitatively predict dose effects that were greater than ten percent of control values (data not shown). The strategy of comparing the effect of chemicals belonging to a "common biological mode of action group" using a toxicologically relevant endpoint (body weight gain) and a standardized dose metric (BMD) in a read-across framework (i.e., between species and chemicals) is consistent with ECHA guidance under REACH (5).

For isoflucypram, the BMD in dogs administered isoflucypram for 52 weeks in the diet was 3.7 mg/kg/day for males and 12.8 mg/kg/day for females. At the high dose tested in the chronic dog study (50 to 60 mg/kg/day), body weight gain was reduced by 30%, indicating that the MTD was exceeded in this group. The BMD in mice fed isoflucypram in

the diet for 78 weeks was 144.3 mg/kg/day in males and 163.4 mg/kg/day. Since the BMD was less than the high dose tested in male (147 mg/kg/day) and female mice (212 mg/kg/day) we conclude that an MTD dose was identified in the carcinogenicity study in mice.

In the chronic rat study on isoflucypram, the BMD was 38.4 mg/kg /day in males after 13 weeks (Table 1) and 51.6 mg/kg/day in females after 52 weeks (Table 2). A BMD could not be calculated after 52 or 104 weeks in males and after 13 and 104 weeks in females because the data could not be fitted by the Hill model at these time points. BMDs could not be calculated for F0 and F1 generation rats fed isoflucypram in the diet for approximately 13 weeks in the multigeneration study.

In chronic rat studies on other pyrazole carboxamide fungicides, BMD's could not be calculated for several of these chemicals (i.e., bixafen and penflufen), particularly for males after 52 and 104 weeks of treatment. Overall, BMD's tended to decline with the age of the animals with lower BMD's after 104 weeks compared to those based on weight gain during the first 13 weeks of the chronic study.

In conclusion, the BMD analysis for isoflucypram indicates that the high dose utilized in the chronic rat study was near to, or at the MTD. The evaluation of additional toxicity endpoints evaluated for the SDHI fungicides also provided additional useful insight into the MTD. For isoflucypram, there was clear evidence of adverse effects in the liver of mice and dogs administered high doses. Rats administered isoflucypram for 4, 13 or 52 or 104 weeks displayed, similar, although sometime less pronounced effects in the liver and secondary effects on the thyroid. Since these changes were on the border between being adaptive responses to being toxicologically adverse effects, the data support the conclusion that high doses of isoflucypram utilized in the chronic rat study and the multigeneration reproduction study approximated the maximum tolerated dose.

References

1) European Chemicals Agency (ECHA). Harmonised Classification and labelling public consultation for Isoflucypram, CAS Number 1255734-28-1; https://echa.europa.eu/harmonised-classification-and-labelling consultation?diss=true&search_criteria_ecnumber=&search_criteria_casnumber=1255734-28-1&search_criteria_name=N-%285-chloro-2-isopropylbenzyl%29-N-cyclopropyl-3-%28difluoromethyl%29-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide%3B+isoflucypram

2) Proposal for Harmonised Classification and Labelling, UK Competent Authority, Chemical Regulation Directorate, Health and Safety Executive, United Kingdom, December, 2018. https://echa.europa.eu/documents/10162/17218/clh_rep_annex_isoflucypram_en.pdf/f21267a7-6223-e994-c869-f8f4b6d32ff6

3) FRAC Code List: Fungicides sorted by mode of action. Fungicide Resistance Action Committee, 2019 http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2019.pdf?sfvrsn=98ff4b9a_2

4) Farber, T.M. (1980) Selection of a maximum tolerated dose (MTD) in oncogenicity studies. A position document of the United States Environmental Agency, Office of Pesticide Programs.

5) European Chemicals Agency (ECHA). Read-Across Assessment Framework (RAAF) ECHA-R-01-EN, March, 2017; https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

Tables and figures: Please see attachment

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Isoflucypram ECHA Public Comment_QS3_Final.pdf

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	5
Comment received				
FR: 10.9 classification and labelling for carcinogenicity Uncertainties remain on the investigation of carcinogenic potential: - The MTD was not reached in the 2-year rat study (in males only slight thyroid histopathological findings were observed at the highest dose tested) - According to OECD 116, for a carcinogenic negative result to be acceptable in a rat carcinogenicity bioassay, survival in the study should ideally be no less than 50% in all groups which was not the case in the 2-year rat study (survival < 50% in all groups). - Structural analogues from the same class of fungicides (i.e.: SDHI pyrazole-carboxamides) induce tumours when tested at higher dose levels (e.g.: sedaxane, pydiflumetofen…)				

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2019	Spain		MemberState	6
Comment received				
No evidence of carcinogenic potential was seen in rat and mice studies. Therefore, the Spanish CA agrees with the dossier submitter that isoflycypram doesn´t warrant classification regarding carcinogenicity.				

Date	Country	Organisation	Type of Organisation	Comment number
24.07.2019	Germany	Bayer AG	Company-Manufacturer	7
Comment received				
With regard to the carcinogenicity (10.9) section, we (Bayer) generally agree with the assessment. In addition to the data presented in the carcinogenicity (10.9) section of the CLH report, we would like to put into context the findings of the 2 year rat carcinogenicity study with the PBTK modeling study of isoflucypram in rat (M-665315-01-1, report attached, data discussed in different comment) and the in vitro characterisation of isoflucypram and the metabolites identified in plasma samples during the 2 year rat carcinogenicity study (M-665264-01-1, report attached, data discussed in different comment). Further, the attachment contains this summary with figures. During the early development of isoflucypram, three specific target organs were identified in the rat. The liver and thyroid were target organs in both sexes and mechanistic investigations indicated that the effects were mediated by CAR/PXR activation, a known toxicological mode of action of SDH inhibitors. The kidney was a target organ in the male rat with the renal effects being identified as precursors of chronic progressive nephropathy. Furthermore, reductions in body weight gain were observed in males (> 30%; no effect in females) in the 28-day study; and in both sexes in the 90-day				

study(12.3% in males and 8.3% in females).

Based on results of the short-term standard and mechanistic studies, we expected to see an increase in liver weight, histopathological changes and possibly hepatocellular adenomas / carcinomas. Further, as liver-mediated thyroid toxicity via CAR-PXR activation is a well-characterized toxicological mode of action in rodents of many compounds (M-665264-01-1, Figure 1) and early mechanistic work confirmed this toxicological mode of action for isoflucypram; we expected to see increased thyroid weight, specific histopathological findings and potentially thyroid adenomas in the long-term study.

Lastly, for the male rats, we expected an increased incidence and severity of histopathological findings associated with chronic progressive nephropathy and potentially an increase in mortality late in the long-term study. This expectation was reinforced by our experiences with another Bayer SDHi fungicide, which had a similar profile for the kidney findings to isoflucypram in the short-term studies. In the chronic rat study done with this molecule shortly before the isoflucypram chronic rat study, excessive mortality led to a decrease of the top dose by 50% in week 85. In that study, chronic progressive nephropathy (sequel to basophilic tubules and hyaline droplets) was noted at the mid- and top dose group in males only.

In accordance with OECD Guidance Document 116, the dose selection for the 2-year carcinogenicity study was a balance between inducing sufficient toxicity and avoiding excessive mortality (see CLH report on Isoflucypram page 30). In males, the high dose for the chronic rat study was set based on doses that in previous studies had caused adverse findings in the kidney. In female rats, the high dose was selected based on achieving some effect on both body weight and liver weight without inducing excessive toxicity for either endpoint. The overall objective was to see appropriate but not excessive toxicity. For more details, we kindly refer to the CLH report.

However, at the end of the 2-year carcinogenicity study there was no increase in any specific tumor type or in the total numbers of benign and malignant neoplasms at any of the doses tested. Furthermore, no neoplastic effects were seen in either males or females at any dose. Besides adaptive effects in females on liver weight, in both sexes on thyroid histopathology, no effects on mortality or body weight gain were observed.

As there were no significant toxicity effects detected at the top dose by the end of the 2-year study, we estimated the isoflucypram plasma exposure that could be expected in rats using a physiologically-based toxico-kinetic (PBTK) modeling approach to get more insight. It is well established that exposure to a chemical substance and the bioavailable amount of the dose are often not identical [Creton et al., 2009]. Describing the toxico-kinetic profile of isoflucypram using PBTK modeling allows extrapolation between doses and administration routes. With this, the internal systemic plasma exposure can be assessed as a function of dose. Using PBTK modeling allows the transfer from a dose-response to an exposure-effect relationship, in terms of internal exposure.

The model shows that an internal exposure plateau (point of non-linearity (grey line; point leading to a saturation effect)) is reached at a dose of 40mg/kg/d for males and 30 mg/kg/d for females (M-665315-01-1, Figure 16). This means that higher doses would not lead to significantly more internal exposure to isoflucypram.

Therefore, it can be concluded that the highest doses tested in the chronic rat study were slightly below (males; about 2-fold higher than 2-year study top dose) or slightly above (females; about 1.5-fold lower than 2-year study top dose) the plateau. Increasing the

dietary concentrations significantly would not have increased the systemic dose of isoflucypram to any appreciable extent. In conclusion, the modeling data reveals that the top dose selected was indeed quite adequate.

Looking at the plasma concentrations in the 2-year study, the metabolites, not isoflucypram, are predominant (M-665264-01-1, Figure 2), indicating that isoflucypram seems to degrade quickly to its major metabolites. Consequently, long-term dietary uptake of isoflucypram leads to internal exposure to the main degradation metabolites (BCS-CX99799 and BCS-CX99799) rather than to internal exposure to isoflucypram. 8

In the standard rat 28 day (SA 11308) and 90 day (SA 12102) studies, both the liver and thyroid were identified as target organs of isoflucypram. In addition, mechanistic studies had determined that the effects observed in these two organs was initiated by CAR/PXR activation. However, contrary to expectations, no adverse effects were observed in the liver or thyroid at the end of the rat 2-year carcinogenicity study.

The absence of any appreciable amounts of isoflucypram in plasma samples taken at various time points during the cancer bioassay, coupled with significant levels of two metabolites in the same samples led to the conduct of preliminary in vitro mechanistic screens to determine the potential of each of the identified plasma metabolites to induce CAR/PXR. A third metabolite, detected in the 24-month samples, was also included in the investigations. The objective of these preliminary in vitro screens was to understand the disparity between the expected and the actual outcome in the long-term rat study.

The in vitro screens confirmed that isoflucypram, is a CAR/PXR agonist (M-665264-01-1, Figure 4) and has the potential to induce CAR/PXR mediated liver effects and liver-mediated thyroid effects. In contrast, the in vitro data indicated that, under the same conditions of test, the metabolites are not CAR/PXR agonists and do not have the potential to induce CAR/PXR mediated liver and liver-mediated thyroid effects (M-665264-01-1, Figure 4). These results were confirmed by the In vitro gene transcription levels using primary cultures of rat hepatocytes (M-665264-01-1, Figure 5).

In summary, short term studies with isoflucypram identified the liver and thyroid as target organs. In addition, mechanistic studies determined that the effects observed in these two organs was initiated by CAR/PXR activation. The kidney was also identified as a target organ in the male rat, with the histopathological findings being associated with chronic progressive nephropathy.

For the 2-year rat study with isoflucypram, we selected the doses in accordance with OECD Guidance Document 116 based on the short-term studies and taking into account available data of related molecules (eg. renal effects by another SDHi). We aimed to induce sufficient toxicity without causing excessive mortality. However, at the end of the 2-year study no significant toxicity was observed.

The PBTK modeling showed that the predicted exposure saturation are in accordance with the top dosage used for the 2 year Rat Carcinogenicity Study. A higher dose would not lead to significantly more internal exposure to isoflucypram. We demonstrated that in the plasma, the metabolites, not isoflucypram, are predominant; indicating that a long-term dietary uptake of isoflucypram leads to exposure to the main degradation metabolites (BCS-CX99799 and BCS-CX99799) rather than exposure to isoflucypram.

The in vitro (gene and receptor) screens confirmed that isoflucypram is a CAR/PXR agonist. In contrast, the in vitro data indicated that the metabolites are not CAR/PXR agonists.

Taking everything together, we hypothesize that we do not see any significant toxicity in the 2-year study because isoflucypram degrades quickly to its metabolites in the rat and the metabolites are not CAR/PXR agonists. The top dose selected for the females was adequate, while from a purely toxicokinetics perspective, a higher dose for male rats would have been reasonable. Nonetheless, a higher dose selection in the male would not lead to significantly more internal exposure to isoflucypram and hence significant toxicity.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment comment_+_reports.zip

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	8
Comment received				
FR: Mammalian cell gene mutation assay In Table 32 page 24, it is mentioned that cytotoxicity was observed which is not supported by the data reported in Table 3.8.2-3 Summary of the results of the HPRT-locus mammalian gene mutation in vitro assay with BCS-CN88460 of the annex (page 171). Indeed, the Relative Survival reported as CE II in this table is not affected even at the highest concentrations. Could you please check? Could you please also report in Table 3.8.2-3 the HCD and the statistical analysis to allow the assessment of the increased mutation frequencies observed in presence of precipitation in order to conclude whether this test is negative or equivocal.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	9
Comment received				
FR: Adverse effects on sexual function and fertility 2-generation study: - As for carcinogenicity, general toxicity was low even at high dose tested in the 2-generation study (MTD not reached) which may compromise the investigation of reproductive toxicity. - A substantial statistically significant increase in the age of vaginal opening (5 days) was observed in F1 generation which is not secondary to lower body weight. The mean age and the mean body weight at completion are outside the HCD range based on Table 38 (the mean and range of HCD mentioned in table 37 for age at vaginal opening is not appropriate since it is the range of individual data) - While it is agreed that F1 females went on to mate successfully and produce the F2 generation, there was a statistical significant shift in gestation length at 1200 ppm (decreased gestation length) Based on the above-mentioned considerations, a classification for reprotoxicity (fertility) may be warranted. Modified rat uterotrophic assay: This assay is a non-GLP, non-guidelined study, a validated method of analysis for				

isoflucypram is not available and in the pubertal assay the number of animal/group was 6 instead of 15 in OPPTS guideline and should not be given much weight.

As regard the absence of effect on vaginal opening in contrast to what was observed in the 2-generation study, besides the above-mentioned limitations it should be highlighted that:
- The 2-generation study covers more sensitive life stage (in utero exposure)
- Only 6 animals/group was used and mean age and weight at vaginal opening was only calculated for the low dose (400 mg/kg bw/d) since the high dose (800 mg/kg bw/d) exceeded the MTD.
- From individual data, vagina was not opened at PND 39 for 1/6 and 2/4 animals at 400 mg/kg bw/d and 800 mg/kg bw/d respectively.

Date	Country	Organisation	Type of Organisation	Comment number
24.07.2019	Germany	Quality Scientific Solutions	Please select organisation type..	10

Comment received

ECHA has invited the public to comment on the hazard classes for isoflucypram (1). The basis for hazard classification and labeling has been summarized by the UK Competent Authority (2). In the section labeled "Deficiencies" in the UK review of the chronic toxicity/carcinogenicity study in the rat and the two-generation reproduction study, the reviewer indicated that the studies complied with the technical relevant guidelines although no adverse findings were noted. At the request of Bayer CropScience, Quality Scientific Solutions, determined if the maximum tolerated dose (MTD) was reached or exceeded in the multigeneration study and the sub-chronic, chronic and lifetime studies in mouse, rat, and dog on the succinate dehydrogenase inhibiting (SDHI) fungicide, isoflucypram (FRAC Code C2 (3)). The hazard identification profile for isoflucypram was examined, and its absorption, distribution, metabolism, and elimination were characterized. Based on this assessment, body weight gain reduction was selected as the primary toxicological indicator of toxicity (4).

Dose-response relationships observed for isoflucypram were compared to dose-response relationships observed for five other pyrazole carboxamide fungicides (bixafen, fluxapyroxad, penflufen, sedaxane, and solatenol) for which there was publicly available data. Benchmark doses (BMDs) were calculated on body weight gain data, a measurement common to all SDHI fungicides. U.S. EPA's Benchmark Dose Software (BMDS, Version 2.6.0.1) was used to fit the Hill model (with summarized means and standard deviations) for "body weight gain (% of control)" versus dose (mmole/kg body weight/day). The Benchmark Response (BMR), which was defined as the point (dose) corresponding to a 10% decrease in body weight gain relative to that of the control group, was calculated when the dose-response could be fitted by the Hill model. BMDs were calculated for isoflucypram based on data from sub-chronic and chronic studies conducted in the rat and the chronic mouse and dog studies and the rat reproduction study. BMDs were calculated on body weight gain data from chronic rat studies reported for each of the five pyrazole carboxamide fungicides to compare the magnitude of these SDHI fungicides to isoflucypram. Average daily doses (mg/kg/day) administered during each interval for which body weight gain was calculated, were converted to a common dose scale (millimole/kg body weight/day) based upon the SDHI's molecular weight. For each SDHI for which there was data, the mmole/kg/day doses were plotted vs. body weight gain to visualize and compare the effect of the SDHI fungicides on body weight gain (Figure 1). As an alternative to BMD analyses, linear regression modeling was used to quantitatively predict dose effects that were greater than ten percent of control values

(data not shown). The strategy of comparing the effect of chemicals belonging to a "common biological mode of action group" using a toxicologically relevant endpoint (body weight gain) and a standardized dose metric (BMD) in a read-across framework (i.e., between species and chemicals) is consistent with ECHA guidance under REACH (5).

For isoflucypram, the BMD in dogs administered isoflucypram for 52 weeks in the diet was 3.7 mg/kg/day for males and 12.8 mg/kg/day for females. At the high dose tested in the chronic dog study (50 to 60 mg/kg/day), body weight gain was reduced by 30%, indicating that the MTD was exceeded in this group. The BMD in mice fed isoflucypram in the diet for 78 weeks was 144.3 mg/kg/day in males and 163.4 mg/kg/day. Since the BMD was less than the high dose tested in male (147 mg/kg/day) and female mice (212 mg/kg/day) we conclude that an MTD dose was identified in the carcinogenicity study in mice.

In the chronic rat study on isoflucypram, the BMD was 38.4 mg/kg /day in males after 13 weeks (Table 1) and 51.6 mg/kg/day in females after 52 weeks (Table 2). A BMD could not be calculated after 52 or 104 weeks in males and after 13 and 104 weeks in females because the data could not be fitted by the Hill model at these time points. BMDs could not be calculated for F0 and F1 generation rats fed isoflucypram in the diet for approximately 13 weeks in the multigeneration study.

In chronic rat studies on other pyrazole carboxamide fungicides, BMD's could not be calculated for several of these chemicals (i.e., bixafen and penflufen), particularly for males after 52 and 104 weeks of treatment. Overall, BMD's tended to decline with the age of the animals with lower BMD's after 104 weeks compared to those based on weight gain during the first 13 weeks of the chronic study.

In conclusion, the BMD analysis for isoflucypram indicates that the high dose utilized in the chronic rat study was near to, or at the MTD. The evaluation of additional toxicity endpoints evaluated for the SDHI fungicides also provided additional useful insight into the MTD. For isoflucypram, there was clear evidence of adverse effects in the liver of mice and dogs administered high doses. Rats administered isoflucypram for 4, 13 or 52 or 104 weeks displayed, similar, although sometime less pronounced effects in the liver and secondary effects on the thyroid. Since these changes were on the border between being adaptive responses to being toxicologically adverse effects, the data support the conclusion that high doses of isoflucypram utilized in the chronic rat study and the multigeneration reproduction study approximated the maximum tolerated dose.

References

1) European Chemicals Agency (ECHA). Harmonised Classification and labelling public consultation for Isoflucypram, CAS Number 1255734-28-1; https://echa.europa.eu/harmonised-classification-and-labelling consultation?diss=true&search_criteria_ecnumber=&search_criteria_casnumber=1255734-28-1&search_criteria_name=N-%285-chloro-2-isopropylbenzyl%29-N-cyclopropyl-3-%28difluoromethyl%29-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide%3B+isoflucypram

2) Proposal for Harmonised Classification and Labelling, UK Competent Authority, Chemical Regulation Directorate, Health and Safety Executive, United Kingdom, December, 2018. https://echa.europa.eu/documents/10162/17218/clh_rep_annex_isoflucypram_en.pdf/f21267a7-6223-e994-c869-f8f4b6d32ff6

3) FRAC Code List: Fungicides sorted by mode of action. Fungicide Resistance Action Committee, 2019 http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2019.pdf?sfvrsn=98ff4b9a_2

4) Farber, T.M. (1980) Selection of a maximum tolerated dose (MTD) in oncogenicity studies. A position document of the United States Environmental Agency, Office of Pesticide Programs.

5) European Chemicals Agency (ECHA). Read-Across Assessment Framework (RAAF) ECHA-R-01-EN, March, 2017; https://echa.europa.eu/documents/10162/13628/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

Tables and figures: Please see attachment

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Isoflucypram ECHA Public Comment_QS3_Final.pdf

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2019	Spain		MemberState	11
Comment received				

Fertility

With regard to fertility, there were no treatment related effects on either mating or fertility indices in either generation of the isoflycypram two generation study. There was a delay in vaginal opening in the F1 offspring of the high dose group (this finding did not reflect lower body weights on a given day of age and the age at vaginal opening was within the historical control range for that laboratory). We agree with the dossier submitter that this finding do not represent an adverse effect of treatment since it did not have a functional consequence on the F1 females in reproducing, and no effects on vaginal opening were seen up to the high gavage dose of 400 mg/kg bw/d for 20 days in a modified rat uterotrophic assay in immature animals which included specific investigations of vaginal opening. Therefore, in the Spanish CA opinion, isoflycypram doesn´t warrant classification regarding fertility.

Development

In the rat isoflycypram developmental study, reduced ossification and visceral variations were observed at high doses. Visceral variations included distended bladder, dilated renal pelvis (unilateral/bilateral) above HCD and present thymic remnant (unilateral / bilateral) (within HCD). Visceral variations occur in the presence of maternal toxicity and only renal pelvis dilation is consistent with findings in the rat reproductive study. However, no kidney, bladder or thymus changes were reported in adult animals exposed in utero in the 2-generation study and there is not impact on the viability of the pups, suggesting that these changes were transient in nature. On overall, the Spanish CA considers that isoflycypram doesn´t warrant classification regarding developmetal effects.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	12
Comment received				

FR: Acute toxicity - Inhalation route page 21
The proposal for classification Acute inhalation toxicity, Category 4 (H332: harmful if inhaled) is supported.

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2019	Spain		MemberState	13
Comment received				
Acute toxicity – inhalation route The lowest LC50 value was 2.209 mg/L (mist aerosol) in females, which lies in between the concentration range of 1.0 and 5.0 mg/L that triggers classification of a mist for acute inhalation toxicity hazard category 4 according to the CLP criteria (EC 1272/2008). Therefore, the Spanish CA support the dossier submitter proposal to classify isoflucypram for acute inhalation toxicity in Category 4 (H332: harmful if inhaled). A harmonised ATE value is also proposed to facilitate consistent classification of mixtures containing Isoflucypram. Taking these data into account, the Spanish CA also supports the ATE of 2.2 mg/L for acute inhalation toxicity.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	14
Comment received				
FR: Skin sensitisation page 24 The proposal for classification Skin Sens., Category 1B (H317: may cause an allergic skin reaction) is supported.				

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2019	Spain		MemberState	15
Comment received				
According to the CLP criteria, a sensitising potential of a substance is identified if a stimulation index of ≥ 3 is obtained in the LLNA. In a LLNA study with isoflucyram, the stimulation index increased in a dose-related manner and was exceeded at the top dose of 50 % (SI = 5.6), with a statistically significant increase also observed at a dose level of 25 % (SI = 2.5). In addition, the calculated EC3 value in this study was 29 % which is indicative of a moderate sensitiser. Therefore, the Spanish CA supports the proposal to classify isoflucypram for skin sensitization in Category 1B (H317: may cause an allergic skin reaction).				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	16
Comment received				
FR: STOT RE page 54-58 Thyroid and liver are identified as target organs in rats. While it is acknowledged that the				

severity of the effects may not warrant classification under CLP regulation, the proposed MoA (CAR-PXR) is however poorly substantiated and uncertainties on this MoA may be further discussed; alternative MoAs have not been considered.
It should be noted that in mice, hepatotoxicity does not seem to result only from a MoA via activation of CAR/PXR. Indeed, increased transaminases levels as well as necrotic focusi support that cytotoxicity is also involved.
The non-relevance to humans of the postulated MoA (CAR-PXR activation) is not supported by compound-specific comparative mechanistic data (human vs rat).

Date	Country	Organisation	Type of Organisation	Comment number
04.07.2019	Spain		MemberState	17
Comment received				

In the available repeated-dose oral toxicity studies, the primary target organ of isoflucypram in all species tested is the liver. Increased liver weight and hypertrophy due to increased enzyme induction was seen in individual studies with rat, mouse and dog at dose levels that would trigger classification for STOT RE, category 2. However, we are in line with the dossier submitter opinion that these effects are adaptive, not consistent across sex or species, often do not progress in severity with study duration and are not sufficiently severe or significant to warrant classification for target organ toxicity.

The Spanish CA agrees with the dossier submitter, that the liver effects observed were via activation of CAR/PXR, a toxicological mode of action which is accepted to be of no relevance for humans. The hepatic enzyme induction work conducted in the rat showed that the pattern of enzyme induction resulting from dietary administration of isoflucypram corresponded to that induced by compounds which activate CAR and / or PXR, and did not correspond to for example PPAR-activating compounds or AhR ligands. The absence of any estrogenic effect in either the uterotrophic assay or the 2-generation reproduction study, as well as any effect on estrogen-sensitive tissues in repeat-dose studies, shows that isoflucypram is not acting on the liver via activation of the estrogen receptor. The general action of statins is to increase hepatocellular proliferation and the levels of Cyp2b and Cyp4a transcript levels without altering serum cholesterol. In contrast, the general action of isoflucypram is to increase serum cholesterol levels, and thus isoflucypram cannot be described to be acting as a statin in experimental animals. There is no indication of any infective conditions in any of the studies, thus infection is not responsible for the increase in liver weight. Although metal overload was not specifically investigated in any study, there were no histopathological findings in any species which would correspond to hepatic accumulation of either iron or copper. No direct measures of apoptosis were undertaken beyond standard histopathological examination, but there was no indication of increased incidence of apoptosis. Thus, in our opinion alternative modes of action which could be responsible for increased liver weight and hepatocellular proliferation can be ruled out.

In conclusion, no adverse effects in any organ were noted that would trigger classification of Isoflucypram as STOT RE, category 1 or category 2.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	France		MemberState	18
Comment received				

FR:
On p.60, the whole system DT50 values reported in the summary table for the water/sediment studies should be 222 to 681 days (RMS calculation retained in the LoEP) instead of 218 to 681 days.

On p.60, the soil DT50 values reported in the summary table for aerobic soil degradation (laboratory) are not consistent with the ones reported in the LoEP. Please check.

Date	Country	Organisation	Type of Organisation	Comment number
26.07.2019	Belgium		MemberState	19

Comment received

Based on the results of the available data, BE CA supports the proposal for environmental classification of the substance isoflucypram with
Aquatic Acute 1, H400 ; M=10
Aquatic Chronic 1, H410; M=1

Editorial comment :
In table 50 (Summary of relevant information on chronic aquatic toxicity) acute data are reported for Skeletonema costatum instead of chronic ones.

PUBLIC ATTACHMENTS
1. comment_+_reports.zip [Please refer to comment No. 7]
2. M-665264-01-1.pdf [Please refer to comment No. 2]
3. M-665315-01-1.pdf [Please refer to comment No. 3]
4. Isoflucypram ECHA Public Comment_QS3_Final.pdf [Please refer to comment No. 4, 10]