CORTEVA™ agriscience

Further Comments of Applicant (Corteva Agriscience) on the CLH report on Proquinazid

and Annex: CLH_REP_ATT_SPS-023780-22_Proquinazid_RAR_08_Volume_3CA_B-6_2022-09-1

02.02.2023

Complimentary to the comments provided via online commenting form, applicant, Corteva Agriscience would like to provide further and more detailed comments to address specific datapoints of and discussions provided in the **CLH Report** & **its annexes** *(correspondence to: Vol 1 and Vol 3 of the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008)).*

Provided comments are in parallel (but selected to *relevance*) with those submitted under Call for comments on the Renewal Assessment Report of Proquinazid (AIR IV) announced by EFSA (EFSA-Q-2018-00520).

Below provided comments also includes the new information generated by applicant and considered "important" for the evaluation and decision making of RAC. Aforementioned new information (study reports, position papers) will be provided to ECHA / RAC review upon request.

CONTENTS

A) Effects on human and animal health 2

- **Absorption, distribution, metabolism and excretion in mammals**
- **Acute toxicity 5**
- **Short-term toxicity 6**
- **Genotoxicity 9**
- **Long-term toxicity and carcinogenicity 25**
- **Reproductive toxicity 29**
- **Neurotoxicity 34**
- **Further toxicological studies**
- **Toxicological end points: ADI, ARfD, AOEL 41**
- **Other comments, proposals for classification 43**

B) List of new Information that Applicant would like to provide 50

Absorption, distribution, metabolism and excretion in mammals			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.6 Effects on human and animal health, Table 2.6.1-1, p.31	**Applicant**: The use of the word 'delayed' in the first row of the table, in the 'remarks' column, requires context. The Applicant proposes the following text: *The overall rate and extent of excretion was a balance of faecal and urinary elimination, of which biliary elimination played a significant role.*	
(2)	Vol. 1, 2.6 Effects on human and animal health, Table 2.6.1-1, p.31	**Applicant**: In the second row of Table 2.6.1-1 the results block is well stated, but the inclusion of the male thyroid is misleading and the applicant requests it be removed from the text. Furthermore, the comment about different strains of rats is inaccurate, as both are Sprague-Dawley derived. *(Applicant comment continues in next column -->)*	The male rat thyroid was declining from 4.4 hours and showed substantial variation at 24 hours (0.291 ± 0.176 µg eq/g) (page 35, Table 4) with a thyroid:plasma ratio of 3.9 ± 3.7 (page 37, Table 6). Close inspection of individual animal thyroid concentrations indicates a wide range of values (i.e., 0.092, 0.356, <LOD, mean ± stn dev. = 0.291 ± 0.176) that suggests sample cross contamination during collection and/or analysis. Furthermore, and importantly, by 48 hours the values were all <LOD (Table 6). Further supporting information can been seen in female rats, where concentrations were at/below the LOD at 25 hours and later.
(3)	Vol. 1, 2.6 Effects on human and animal health, Table 2.6.1-1, p.31	**Applicant**: In the third row of Table 2.6.1-1, the Applicant disagrees with the comment pertaining to differences *in vitro* would carry over *in vivo*. *(Applicant comment continues in next column -->)*	The applicant considers it a scientific overreach to assume that species differences *in vitro* would directly scale a difference *in vivo*, rat vs human. The applicant proposes the following wording as more appropriate: *Despite the apparent lower overall metabolism in the human, similar metabolites were observed across species. Metabolites in human hepatocytes were consistently observed in the other species.*
(4)	Vol 1, 2.6 Effects on human and animal health, Figure 2.6.1-1, p.33	**Applicant**: The Applicant disagrees with the changing of the proposed *in vivo* metabolic pathway for the rat from that previously agreed. *(Applicant comment continues in next column -->)*	The pathway shown is from the single dose ADME study ([redacted]3989), and it was upgraded in the repeated dose ADME [redacted]-1075). The metabolic pathway depicted in [redacted]-1075 should be used as the most recent *in vivo* version for the rat. A note could be added that the [redacted]-1075 pathway is consistent with the single dose *in vivo* study [redacted]3989) and the profile observed in the *in vitro*

Absorption, distribution, metabolism and excretion in mammals			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			comparative study ([redacted] 47158) shown in Figure 2.6.1-2 immediately below in the 1.
(4)	Vol. 1, 2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s), p. 35	**Applicant**: The Applicant requests revision of the text in the first paragraph of p.35 in relation to the tissues mentioned (bone marrow, reproductive and male thyroid), as this is inconsistent with the summary at the beginning of the section. This is also reflected on p.12 of the B6. *(Applicant comment continues in next column -->)*	The Applicant proposes the following text: "Following oral administration of proquinazid, extensive tissue distribution was observed. Potential for preferential tissue uptake was low."
(5)	Vol. 3, CA B.6, B.6.1.1(02), Absorption, distribution, metabolism and excretion by oral route, p.20	**Applicant**: First paragraph on page 21, the symbol has been lost in the last sentence in relation to the enzyme, this should be a beta symbol.	
(6)	Vol. 3, CA B.6, Absorption, distribution, metabolism and excretion by oral route, Table 6.1.1-9, p.11	**Applicant**: In the second paragraph on p.10 the applicant requests a revision of the statement pertaining to the fraction of partitioning of proquinazid between plasma and blood as it is not wholly supported by the data in Table 6.1.1-9, p.11. *(Applicant comment continues in next column -->)*	The Applicant suggests that based on the data in Table 6.1.1-9, (p.11), that the fraction should be revised from "2/3" to "3/4 to 4/5th (0.72 - 0.8)". Applicant notes that this range represents an overall minor effect explained by the protein and/or lipid content of the red bleed cell components in whole blood.
(7)	Vol. 3, Table 6.1.1-14, Absorption, distribution, metabolism and excretion by oral route, p.14-15	**Applicant**: The Applicant requests that the means and standard deviations be added to Table 6.1.1-14 (P.14-15).	

Absorption, distribution, metabolism and excretion in mammals			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		Presentation of individual animal results in a summary rather than Mean ± SD, or without also including the Mean ± SD is not appropriate since Mean summary data are referred to in the main dossier text. The Table should be revised to include the Mean ± SD.	
(8)	Vol. 3, CA B.6, B.6.1.1(02), Absorption, distribution, metabolism and excretion by oral route, p.26.	**Applicant**: The Applicant requests the inclusion of the repeat dose proposed metabolic pathway at the end of the study summary for B.6.1.1(02). The repeat dose proposed metabolic pathway [redacted]-1075) for the rat is the most recent metabolic profile for proquinazid and is an up-grade from that included in the single dose study [redacted] 3989) (B.6.1.1(01)). Hence, the [redacted]-1075 pathway should be used as the overall metabolic profile for the rat at the end of this second study summary and consequently in Vol. 1. This would maintain consistency with the previous registration.	
(9)	Vol. 3, CA B.6, B.6.1.1(02), Absorption, distribution, metabolism and excretion by oral route, p.27.	**Applicant**: In the second from bottom paragraph of the "Assessment and conclusion by RMS" box, the Applicant disagrees with the inclusion of the thyroid and the statement pertaining to levels being "5 times higher in thyroid" relative to plasma. *(Applicant comment continues in next column -->)*	The male rat thyroid showed substantial variation at 24 hours (0.291 ± 0.176 µg eq/g) (page 35, Table 4) with a thyroid:plasma ratio of 3.9 ± 3.7 (page 37, Table 6). Close inspection of individual animal thyroid concentrations indicates a wide range (i.e., 0.092, 0.356, <LOD) that suggests sample cross contamination during collection and/or analysis. More importantly, the individual values were all <LOD by 48 hours (Table 6). In the female rats, thyroid concentrations were at/below the LOD at 25 hours and later. The apparent higher ratio in the male thyroid vs plasma appears to be due to analytical variation.

Absorption, distribution, metabolism and excretion in mammals			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(10)	Vol. 3, CA B.6, B.6.1.1(03), Multispecies in vitro metabolism in hepatocytes, p.28.	**Applicant**: Applicant requests that "Not Applicable" should be included in the deviations box given there is no guideline for which this study can be compared to.	
(11)	Vol. 3, CA B.6, B.6.1.1(03), Multispecies in vitro metabolism in hepatocytes, p.35.	**Applicant**: The applicant disagrees with equating the cell viability to the rate of 14C-proquinazid metabolism, and requests the RMS to revise the text accordingly. *(Applicant comment continues in next column -->)*	The Applicant disagrees to the equation of cell viability (Trypan Blue exclusion by microscopic observation) with the rate of 14C-proquinazid metabolism. Trypan Blue is used to determine and set the number of viable cells at the start of incubation. It was also measured at the end of incubation and well maintained except for the mouse. A more appropriate comparison is to look at the positive control data for 7-Ethoxycoumarin which measures phase 1 and phase 2 biotransformation. The total ECOD rate in human was lower than the other species, consistent with differences in how donor tissues are sourced for human vs non-human species (Table 2[redacted]-47158). Overall, the positive control ECOD confirms suitability of the test system while cell viability is performed to make sure the number of viable cells used in each incubation is similar at the beginning of the experiment.

Acute toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.6.2.1.1, Short summary and overall relevance of the provided	**Applicant**: The endpoint for females (page 36) should be '=4846 mg/kg body weight' rather than greater than. The preceding table (Table 2.6.2.1-1.) is correct. Regardless, the classification is not impacted, and the	

Acute toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	information on acute oral toxicity, p.36	Applicant supports the concluding statements in sections 2.6.2.1.2. and 2.6.2.1.3. This appears to be a carryover from the MCA5, the Applicant apologises for the error and respectfully requests the text be updated.	
(2)	Vol. 3, CA B.6, B.6.2.4 Skin irritation	**Applicant:** In the second line of the methods paragraph the 2 of '6 cm2' should be a superscript number (p. 40).	
(3)	Vol. 3, CA B.6, B.6.2.7, Phototoxicity p.43-45	**Applicant:** Thank you to the RMS for the inclusion of the context and communications on this topic. The applicant is in support of the RMS's position.	

Short-term toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 3, CA B.6, B.6.3.2(03) Subchronic toxicity study 90-day feeding study in dogs.	**Applicant** apologises for the lack of mg/kg bw/day equivalents in the MCA5[redacted]-1997-00157 summary that were inadvertently carried over into the B6. The applicant respectfully requests they be included in the study summary. *(Applicant comment continues in next column -->)*	For ease of reference the mg/kg bw/day equivalents are as follows:

Short-term toxicity

No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			Group / Theoretical Dietary Level (ppm) / Average Calculated Test Substance Consumption (mg/kg/day) Males / Females Control / 0 / 0 / 0 500 ppm / 500 / 17 / 18 2000 ppm / 2000 / 62 / 56 4000/3000 ppm / 4000 (weeks 0-1 to 4-5) / 87 / 93 3000 (weeks 6-8 to 12-13) / 87 / 96 This is taken from p.41 of Study: [redacted]-1997-00157.
(2)	Vol. 3, CA B.6, B.6.3.2(03) Subchronic toxicity study 90-day feeding study in dogs, p.96 And, Vol. 1, Table 2.6.3.1-1. Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure), p.53-4	**Applicant:** The Applicant respectfully disagrees with the conclusion on page 96 that liver weight changes represent an adverse effect, as these liver weight increases were not associated any correlative findings indicative of liver injury or altered liver function and were not present in the subsequent longer-term study conducted at similar dose levels. *(Applicant comment continues in next column -->)*	Absent correlative changes in clinical chemistries or histopathology, increased liver weight is generally considered as adaptive rather than adverse (Hall et al., 2012; Foster et al., 2020; Pandri et al, 2017). In addition, there was no indication of functional effects on the liver and as such, the liver weight effects in isolation do not meet accepted criteria for adversity (WHO/IPCS, 2004; Kerlin et al., 2015; Palazzi et al, 2016; Pandri et al, 2017). The non-adversity of the liver weight findings is also indicated by the lack of liver weight changes in the 1-year study in dogs at comparable dose levels.

Short-term toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(3)	Vol. 3, CA B.6, B.6.3.2(04) Chronic toxicity study one-year capsule study in dogs, p.129 Vol. 1, Table 2.6.3.1-1. Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure), p.55 And, Vol. 1, 2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity), p.73-4	**Applicant:** The Applicant proposes that any summary statement on adversity at least note that these findings occurred at doses in excess of an MTD and thus are not/may not be indicative of a primary effect. *(Applicant comment continues in next column -->)*	As noted by the RMS, at the 60 and 180 mg/kg/day dose levels, there was significant systemic toxicity. This included clinical signs of vomiting and salivation, marked effects on body weight gain (31% and 41% in the 60 and 180 mg/kg/day males, respectively), and at 180 mg/kg/day, thymic necrosis. The systemic toxicity at these dose levels precludes assessment of a primary effect on the testes/epididymides, and as noted in the study report, most likely represent secondary effects. This conclusion is further supported by the absence of primary testicular toxicity in other species, Should the RMS still consider these findings relevant for highlighting, then the Applicant propose that any summary statement on adversity should at least note that these findings occurred at doses in excess of an MTD and thus are not/may not be indicative of a primary effect.
(4)	Vol. 3, CA B.6, B.6.3.2(05) Oral 90-day study in mice, p.138	**Applicant** proposes that any concluding statement in relation to the significance of the mammary gland findings note severe systemic toxicity seen at dose levels where the mammary gland changes were observed.	The Applicant would like to highlight that the findings in the mammary gland of both males and females occurred at doses causing clear and severe stress (as indicated by bodyweight effects, thymic necrosis) and marked liver toxicity (fatty change and necrosis). As noted in the study report (and by the RMS elsewhere), there was marked loss of subcutaneous fat, including the

Short-term toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		(Applicant comment continues in next column -->)	mammary gland fat pad which very likely impacted the histopathological appearance of the mammary ducts. The Applicant stresses that the severe systemic toxicity at 7000 and 12000 and the associated secondary changes in reproductive/mammary tissue precludes any meaningful assessment of primary adverse effects in those organs/tissues.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted] 1600 [redacted] (1998) p.85	**Applicant**: Please correct the "Relevant information" column to indicate the E.coli strain used was WP2 uvra (pKM101). This is relevant to the text and positive controls table. Currently the text suggests WP2 uvrA was used.	
(2)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted] -1997- 00040 [redacted] (1997) p.86	**Applicant**: Please correct the "Relevant information" column to indicate the E.coli strain used was WP2 uvra (pKM101). This is relevant to the text and positive controls table. Currently the text suggests WP2 uvrA was used.	

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(3)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted] 1099-96, Revision No. 1 [redacted] (1999) p.86-87	**Applicant:** The "Observations and Results" column text states that the study may have limited sensitivity due to the high levels of toxicity and relatively low positive control responses. The applicant agrees the study has limited acceptability due to the inadequate study design (a lack of a continuous exposure in the absence of S9 and only 200 metaphases scored for aberrations). However, the applicant does not agree that the sensitivity of the test has been compromised by either toxicity or low positive control responses. *(Applicant comment continues in next column -->)*	On only two occasions in the repeat assay did high toxicity limit the number of metaphases that could be scored (5000 µg/mL treatments at the 24-hour sample time both with and without metabolic activation). In both instances, the target of 200 metaphases was achieved at three lower concentrations, indicating that the high cytotoxicity did not reduce the sensitivity of the test. In all other conditions in the repeat test and in all conditions of the initial test, the target of 200 metaphases was achieved at each concentration examined. With respect to the positive controls, all positive control responses were within the laboratory's HCD and met the OECD 473 (2016) requirement for a clear response that would not immediately reveal the identity of the coded slides to the reader.
(4)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted] 1100-96, Revision No. 1 [redacted] (1999) p.88	**Applicant:** The maximum dose tested is identified as a deviation, however, in the presence of dose limiting animal toxicity there is no requirement to achieve 2000 mg/kg. The applicant respectfully suggests that this is not a deviation.	
(5)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted] 1100-96, Revision No. 1 [redacted] (1999) p.88	**Applicant:** It is correct that this study was deficient in the number of immature erythrocytes scored for micronuclei (2000 instead of 4000). However, the MCA5 also identified a deviation in the number of total erythrocytes scored for immature erythrocyte frequency. This second deviation was an error. OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored for bone marrow. In this study 1000 total erythrocytes were scored and	The applicant respectfully requests that the second part of this deviation is removed from both the first and fourth columns. 1000 erythrocytes were scored to determine the frequency of immature erythrocytes in the bone marrow smears. According to OECD TG474, 2000 erythrocytes should be scored where assessments are performed in peripheral blood. For this study, analysis was performed in bone marrow.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		therefore this study does not deviate from the TG with regards assessment of erythrocyte frequency. *(Applicant comment continues in next column -->)*	
(6)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted] 1100-96, Revision No. 1 [redacted] (1999) p.88	**Applicant:** In the Observations and results column the applicant respectfully disagrees that there was no proof of bone marrow exposure. It is correct that there was no specific blood or plasma analysis in the study, but this is only required by OECD TG 474 where warranted and where other exposure data do not exist. There are lines of evidence that support a proof of exposure assessment for this study. *(Applicant comment continues in next column -->)*	According to Hardy et al. 2017, (EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113) there are several lines of evidence that may be used to demonstrate bone marrow exposure, one of which is a specific blood or plasma analysis performed concurrently with the study. However, it may be possible to conclude there was bone marrow exposure using other lines of evidence. The following lines of evidence are considered to support the conclusion of bone marrow exposure in this study: • There was evidence of bone marrow toxicity (as seen by a reduction in frequency of immature cells) in female mice • There was animal mortality at the maximum dose tested (1440 mg/kg) in female mice. In the toxicity assay higher doses (2000 mg/kg and above) resulted in mortality in male and female mice • The clinical signs observed in the study were indicative of effects on the central nervous system (lethargy, ataxia), which would usually only occur if the substance is bioavailable • In a single oral dose ADME study in rats, using radiolabelled proquinazid (B.6.1.1(01)), radiolabel was found in bone marrow at similar levels to that plasma and blood In a more recent mouse micronucleus test B.6.4.2(01), proquinazid was detected at >500 ng/mL in the plasma of male and female mice that received a

Genotoxicity			
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			single oral gavage dose of 500 mg/kg (blood sampled 4 hours after dose administration).
(7)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted]-1791 [redacted] (1999) p.88-89	**Applicant:** In the "Observations and results" column, the maximum dose tested is identified as a deviation, however, in the presence of dose limiting animal toxicity there is no requirement to achieve 2000 mg/kg. The applicant respectfully suggests that this is not a deviation.	
(8)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted]-1791 [redacted] (1999) p.88-89	**Applicant:** It is correct that this study was deficient in the number of immature erythrocytes scored for micronuclei (2000 instead of 4000). However, the MCA5 also identified a deviation in the number of total erythrocytes scored for immature erythrocyte frequency. This second deviation was an error. OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored for bone marrow. In this study 1000 total erythrocytes were scored and therefore this study does not deviate from the TG with regards assessment of erythrocyte frequency. *(Applicant comment continues in next column -->)*	The applicant respectfully requests that the second part of this deviation is removed from both the first and fourth columns. 1000 erythrocytes were scored to determine the frequency of immature erythrocytes in the bone marrow smears. According to OECD TG474, 2000 erythrocytes should be scored where assessments are performed in peripheral blood. For this study, analysis was performed in bone marrow.
(9)	Vol. 1, 2.6.4, Table 2.6.4-1. Reference [redacted]-1791 [redacted] (1999) p.88-89	**Applicant:** In the Observations and Results column the applicant respectfully disagrees that there was no proof of bone marrow exposure. It is correct that there was no specific blood or plasma analysis in the study, but this is only required by OECD TG 474 where warranted and where other exposure data do	According to Hardy et al. 2017, (EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113) there are several lines of evidence that may be used to demonstrate bone marrow exposure, one of which is a specific blood or plasma analysis performed concurrently with the study.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		not exist. There are lines of evidence that support a proof of exposure assessment for this study. *(Applicant comment continues in next column -->)*	However, it may be possible to conclude there was bone marrow exposure using other lines of evidence. The following lines of evidence are considered to support the conclusion of bone marrow exposure in this study: • There was evidence of bone marrow toxicity (as seen by a reduction in frequency of immature cells) in female mice • There was animal mortality at the maximum dose tested (1440 mg/kg) in female mice. In the toxicity assay higher doses (2000 mg/kg and above) resulted in mortality in male and female mice • The clinical signs observed in the study were indicative of effects on the central nervous system (lethargy, ataxia), which would usually only occur if the substance is bioavailable • In a single oral dose ADME study in rats, using radiolabelled proquinazid (B.6.1.1(01)), radiolabel was found in bone marrow at similar levels to that plasma and blood In a more recent mouse micronucleus test B.6.4.2(01), proquinazid was detected at >500 ng/mL in the plasma of male and female mice that received a single oral gavage dose of 500 mg/kg (blood sampled 4 hours after dose administration).
(10)	Vol. 1 2.6.4.1, Short Summary	**Applicant:** Please update the short summary incorporating the above comments regarding study deviations.	
(11)	Vol. 3, CA B.6, B.6.4.1(01), CA 5.4.1, Bacterial reverse mutation test. p.152	**Applicant:** Deviations from current test guideline. In error the MCA5 identified a lack of analysis on the formulated test material as a deviation from OECD	

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		471 (1997). This is incorrect as neither the 1997 or 2020 versions of OECD 471 stipulate a requirement for analysis of the formulated test material. The applicant respectfully requests that this deviation is removed. Analysis of formulated test material is a GLP requirement, however, it is not a requirement of OECD 471 and therefore there are no deviations from current test guideline for this study.	
(12)	Vol. 3, CA B.6, B.6.4.1(01), CA 5.4.1, Bacterial reverse mutation test. p.152	**Applicant:** Please amend the title of the table to read "Positive controls" as no information on the Negative (Vehicle) control is presented in the table. The negative (vehicle) control is documented with the test substance information	
(13)	Vol. 3, CA B.6, B.6.4.1(01), CA 5.4.1, Bacterial reverse mutation test. p.152	**Applicant:** Please present the E coli strain as WP2 uvrA (pKM101) in the bacterial strain column of the Positive controls table. This will remove any confusion as to whether WP2 uvrA or WP2 uvrA (pKM101) was tested.	
(14)	Vol. 3, CA B.6, B.6.4.1(01), CA 5.4.1, Bacterial reverse mutation test. p.153-154	**Applicant:** In Tables 6.4.1(01)-1 and 6.4.1(01)-2, please present the E coli strain as WP2 uvrA (pKM101).	

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		This will remove any confusion as to whether WP2 uvrA or WP2 uvrA (pKM101) was tested.	
(15)	Vol. 3, CA B.6, B.6.4.1(01), CA 5.4.1, Bacterial reverse mutation test. p.155	**Applicant:** Please amend the first sentence of the last paragraph in line with the comment above **(Genotoxicity Comment (11) Vol. 3, B.6.4.1(01), CA 5.4.1, Bacterial reverse mutation test. P152).** *(Applicant comment continues in next column à)*	Suggest the last paragraph is amended to: No analysis was performed on formulated test material~~, although this is stipulated in the current OECD 471 guidelines~~. However, based on the study report and the certification of the test facility, the RMS assume that any variations in the concentrations of the test material will not have affected the interpretability of the results from this study.
(16)	Vol. 3, CA B.6, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. p.155	**Applicant:** Deviations from current test guideline. In error the MCA5 identified a lack of analysis on the formulated test material as a deviation from OECD 471 (1997). This is incorrect as neither the 1997 or 2020 versions of OECD 471 stipulate a requirement for analysis of the formulated test material. The applicant respectfully requests that this deviation is removed. *(Applicant comment continues in next column à)*	Analysis of formulated test material is a GLP requirement, however, it is not a requirement of OECD 471 and therefore there are no deviations from current test guideline for this study.
(17)	Vol. 3, CA B.6, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. p.156	**Applicant:** Please amend the title of the table to read "Positive controls" as no information on the Negative (Vehicle) control is presented in the table.	

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		The negative (vehicle) control is documented with the test substance information	
(18)	Vol. 3, CA B.6, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. p.156	**Applicant:** Please present the E coli strain as WP2 uvrA (pKM101) in the bacterial strains column of the Positive controls table. This will remove any confusion as to whether WP2 uvrA or WP2 uvrA (pKM101) was tested.	
(19)	Vol. 3, CA B.6, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. p.157	**Applicant:** The closing bracket is missing from Line 2 of Paragraph 1: "(TA100 and WP2 *uvrA* only)	
(20)	Vol. 3, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. p.158	**Applicant:** In Tables 6.4.1(02)-1 and 6.4.1(02)-2, please present the E coli strain as WP2 uvrA (pKM101). This will remove any confusion as to whether WP2 uvrA or WP2 uvrA (pKM101) was tested.	
(21)	Vol. 3, CA B.6, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. p.159	**Applicant:** Please amend the first sentence of the last paragraph in line with the comment above **(Genotoxicity Comment (16) Vol. 3, B.6.4.1(02), CA 5.4.1, Bacterial reverse mutation test. P155).** *(Applicant comment continues in next column -->)*	Suggest the last paragraph is amended to: No analysis was performed on formulated test material~~, although this is stipulated in the current OECD 471 guidelines~~. However, based on the study report and the certification of the test facility, the RMS assume that any variations in the concentrations of the test material will not have affected the interpretability of the results from this study.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(22)	Vol. 3, CA B.6, B.6.4.1(03), CA 5.4.1, Bacterial reverse mutation test. p.159	**Applicant:** Deviations from current test guideline. In error the MCA5 identified a lack of analysis on the formulated test material and positive controls as a deviation from OECD 471 (1997). This is incorrect as neither the 1997 or 2020 versions of OECD 471 stipulate a requirement for analysis of the formulated test material or positive controls. The applicant respectfully requests that this deviation is removed. *(Applicant comment continues in next column -->)*	Analysis of formulated test material is a GLP requirement, however, it is not a requirement of OECD 471 and therefore there are no deviations from current test guideline for this study.
(23)	Vol. 3, CA B.6, B.6.4.1(03), CA 5.4.1, Bacterial reverse mutation test. p.160	**Applicant:** The text in Paragraph 2 (preceding the Positive Controls Table) summarises both trials, however, only the details of Trial 1 are documented. Trial 2 used 3 plates/concentration not 2 plates/concentration, and precipitation was seen starting at 667 µg/plate with and without S9 not at 333 µg/plate with S9.	
(24)	Vol. 3, CA B.6, B.6.4.1(03), CA 5.4.1, Bacterial reverse mutation test. p.160	**Applicant:** The text "Negative (Vehicle) and" should be deleted from the title of the table as the table only details the Positive Controls. The negative (vehicle) control is documented with the test substance information	

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(25)	Vol. 3, CA B.6, B.6.4.1(03), CA 5.4.1, Bacterial reverse mutation test. p.161	**Applicant:** Please delete the erroneous text at the end of the last sentence of Paragraph 1 (Toxicity-Mutation Test). The last sentence should read: The toxicity-mutation test used duplicate plates for each concentration.~~ma~~	
(26)	Vol. 3, CA B.6, B.6.4.1(03), CA 5.4.1, Bacterial reverse mutation test. p.164	**Applicant:** Please amend the first sentence of the last paragraph in line with the comment above **(Genotoxicity Comment (22) Vol. 3, B.6.4.1(03), CA 5.4.1, Bacterial reverse mutation test. P159).** *(Applicant comment continues in next column -->)*	Suggest the last paragraph is amended to: No analysis was performed on formulated test material~~, although this is stipulated in the current OECD 471 guidelines~~. However, based on the study report and the certification of the test facility, the RMS assume that any variations in the concentrations of the test material will not have affected the interpretability of the results from this study.
(27)	Vol. 3, CA B.6, B.6.4.1(04), CA 5.4.1, Chromosomal abnormalities in mammalian cells. p.165	**Applicant:** The text "Negative (Vehicle) and" should be deleted from the title of the table as the table only details the Positive Controls.	The negative (vehicle) control is documented with the test substance information
(28)	Vol. 3, CA B.6, B.6.4.1(04), CA 5.4.1, Chromosomal abnormalities in mammalian cells. p.170	**Applicant:** The RMS assessment and conclusion states that the study may have limited sensitivity due to the high levels of toxicity and relatively low positive control responses. The applicant agrees the study has limited acceptability due to the inadequate study design (a lack of a continuous exposure in the absence of S9 and only 200 metaphases scored for	On only two occasions in the repeat assay did high toxicity limit the number of metaphases that could be scored (5000 µg/mL treatments at the 24-hour sample time both with and without metabolic activation). In both instances, the target of 200 metaphases was achieved at three lower concentrations, indicating that the high cytotoxicity did not reduce the sensitivity of the test. In all other conditions in the repeat test and in all conditions of the initial test, the target of 200 metaphases was achieved at each concentration examined.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		aberrations). However, the applicant does not agree that the sensitivity of the test has been compromised by either toxicity or low positive control responses. *(Applicant comment continues in next column -->)*	With respect to the positive controls, all positive control responses were within the laboratory's HCD and met the OECD 473 (2016) requirement for a clear response that would not immediately reveal the identity of the coded slides to the reader.
(29)	Vol. 3, CA B.6, B.6.4.1(05), CA 5.4.1, Mammalian cell assay for gene mutation. p.171	**Applicant:** The text "Negative (Vehicle) and" should be deleted from the title of the table as the table only details the Positive Controls. The negative (vehicle) control is documented with the test substance information	
(30)	Vol. 3, CA B.6, B.6.4.1(06), CA 5.4.1, Mouse lymphoma cells assay. p.178	**Applicant:** Deviations from current test guideline. In error the MCA5 indicated that the study report did not specify cloning efficiency. However, this was erroneous as cloning efficiency (CE) is reported in Appendices 2-5 of the study report. The applicant respectfully requests that this deviation is removed. The study report presents CE as % viability (%V) in the results tables of the appendices.	
(31)	Vol. 3, CA B.6, B.6.4.1(06), CA 5.4.1, Mouse lymphoma cells assay. p.178	**Applicant:** Deviations from current test guideline. In error the MCA5 indicated that in the study an insufficient number of cells were scored. However, this is a misleading statement as under OECD TG490 there is no requirement to score a required number	OECD TG490 recommends treating at least 6 x10^6 cells and to maintain sufficient cells at all stages to achieve at least 10 spontaneous mutants. From the report at least 1x 10^7 cells were treated in the 3 h experiments. Although only 4 x10^6 cells were treated in the 24 h experiments, all experiments (3 and 24 h) plated more than 700,000 cells for mutant expression and cloning, and

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		of cells. There is a requirement to treat a specific number of cells and to maintain adequate numbers of cells so as to maintain at least 10 spontaneous mutants per culture. The number of spontaneous mutants was achieved in this study. The applicant respectfully requests that this deviation is removed. *(Applicant comment continues in next column -->)*	>10 mutant clones were found per culture. Therefore, this is not a relevant deviation for this study.
(32)	Vol. 3, CA B.6, B.6.4.2(01), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.187	**Applicant:** It is correct that this study was deficient in the number of immature erythrocytes scored for micronuclei (2000 instead of 4000). However, the MCA5 also identified a deviation in the number of total erythrocytes scored for immature erythrocyte frequency. This second deviation was an error. OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored for bone marrow. In this study 1000 total erythrocytes were scored and therefore this study does not deviate from the TG with regards assessment of erythrocyte frequency. The applicant respectfully requests that the second part of this deviation is removed. *(Applicant comment continues in next column -->)*	1000 erythrocytes were scored to determine the frequency of immature erythrocytes in the bone marrow smears. According to OECD TG474, 2000 erythrocytes should be scored where assessments are performed in peripheral blood. For this study, analysis was performed in bone marrow.
(33)	Vol. 3, CA B.6, B.6.4.2(01), CA 5.4.2, In vivo studies in	**Applicant:** On reflection, the applicant considers the deviations wording added to the MCA 5 as inaccurate	The applicant respectfully requests that this deviation is removed or amended to reflect the fact that although no blood or plasma sample(s) was retained,

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.187	and therefore misleading. Proof of bone marrow exposure was deemed necessary in the study, but as it was considered that there were several lines of evidence to support bone marrow exposure, a specific blood or plasma analysis was not deemed necessary. *(Applicant comment continues in next column -->)*	this was deemed unnecessary as bone marrow exposure was demonstrated using other lines of evidence. Although no blood or plasma sample(s) was retained, this was deemed unnecessary as bone marrow exposure was considered to have occurred (see comment 19 for further details). This is in line with OECD TG 474 recommendations, which recommends retention of a blood or plasma sample "where warranted and where other exposure data do not exist".
(34)	Vol. 3, CA B.6, B.6.4.2(01), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.192	**Applicant:** Please amend bullet (2) with respect to the number of erythrocytes enumerated for erythrocyte frequency. For immature erythrocyte frequency, OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored. In this study bone marrow study, 1000 erythrocytes were scored.	
(35)	Vol. 3, CA B.6, B.6.4.2(01), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.192	**Applicant:** Please amend bullet (3) with respect to the proof of bone marrow exposure. It is correct that there was no specific blood or plasma analysis in the study, but this is only required by OECD TG 474 where warranted and where other exposure data do not exist. There are lines of evidence that support a proof of exposure assessment for this study. *(Applicant comment continues in next column -->)*	According to Hardy et al. 2017, (EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113) there are several lines of evidence that may be used to demonstrate bone marrow exposure, one of which is a specific blood or plasma analysis performed concurrently with the study. However, it may be possible to conclude there was bone marrow exposure using other lines of evidence. The following lines of evidence are considered to support the conclusion of bone marrow exposure in this study: • There was evidence of bone marrow toxicity (as seen by a reduction in frequency of immature cells) in female mice

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			• There was animal mortality at the maximum dose tested (1440 mg/kg) in female mice. In the toxicity assay higher doses (2000 mg/kg and above) resulted in mortality in male and female mice • The clinical signs observed in the study were indicative of effects on the central nervous system (lethargy, ataxia), which would usually only occur if the substance is bioavailable • In a single oral dose ADME study in rats, using radiolabelled proquinazid (B.6.1.1(01)), radiolabel was found in bone marrow at similar levels to that plasma and blood • In a more recent mouse micronucleus test B.6.4.2(01), proquinazid was detected at >500 ng/mL in the plasma of male and female mice that received a single oral gavage dose of 500 mg/kg (blood sampled 4 hours after dose administration).
(36)	Vol. 3, CA B.6, B.6.4.2(02), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.193	**Applicant:** It is correct that this study was deficient in the number of immature erythrocytes scored for micronuclei (2000 instead of 4000). However, the MCA5 also identified a deviation in the number of total erythrocytes scored for immature erythrocyte frequency. This second deviation was an error. OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored for bone marrow. In this study 1000 total erythrocytes were scored and therefore this study does not deviate from the TG with regards assessment of erythrocyte frequency. The applicant respectfully requests that the second part of this deviation is removed.	1000 erythrocytes were scored to determine the frequency of immature erythrocytes in the bone marrow smears. According to OECD TG474, 2000 erythrocytes should be scored where assessments are performed in peripheral blood. For this study, analysis was performed in bone marrow.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		(Applicant comment continues in next column -->)	
(37)	Vol. 3, CA B.6, B.6.4.2(02), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.193	**Applicant:** On reflection, the applicant considers the deviations wording added to the MCA 5 as inaccurate and therefore misleading. Proof of bone marrow exposure was deemed necessary in the study, but as it was considered that there were several lines of evidence to support bone marrow exposure, a specific blood or plasma analysis was not deemed necessary. The applicant respectfully requests that this deviation is removed or amended to reflect the fact that although no blood or plasma sample(s) was retained, this was deemed unnecessary as bone marrow exposure was demonstrated using other lines of evidence. *(Applicant comment continues in next column -->)*	Although no blood or plasma sample for potential analysis of proquinazid concentration was retained in this study this does not constitute a deviation as OECD TG474 recommends sample retention "where warranted and where other exposure data do not exist". See comment 23 for the lines of evidence that support bone marrow exposure.
(38)	Vol. 3, CA B.6, B.6.4.2(02), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. P196	**Applicant:** Please amend bullet (2) with respect to the number of erythrocytes enumerated for erythrocyte frequency. For immature erythrocyte frequency, OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored. In this study 1000 erythrocytes were scored.	

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(39)	Vol. 3, CA B.6, B.6.4.2(02), CA 5.4.2, In vivo studies in somatic cells - Metaphase or micronucleus analysis rodent bone marrow. p.196	**Applicant:** Please amend bullet (3) with respect to the proof of bone marrow exposure. It is correct that there was no specific blood or plasma analysis in the study, however, there are lines of evidence that support a proof of exposure assessment. *(Applicant comment continues in next column -->)*	In accordance with Hardy et al. 2017, (EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113) the following lines of evidence are considered to support the conclusion of bone marrow exposure in this study: • There was animal mortality at the maximum dose tested (1440 mg/kg in female mice; 2000 mg/kg in male mice). • The clinical signs observed in the study were indicative of effects on the central nervous system (lethargy, ataxia, abnormal gait), which would usually only occur if the substance is bioavailable • In a single oral dose ADME study in rats, using radiolabelled proquinazid (B.6.1.1(01)), radiolabel was found in bone marrow at similar levels to that plasma and blood • In a more recent mouse micronucleus test B.6.4.2(01), proquinazid was detected at >500 ng/mL in the plasma of male and female mice that received a single oral gavage dose of 500 mg/kg (blood sampled 4 hours after dose administration).
(40)	Vol. 3, CA B.6, B.6.4 Genotoxicity And, Vol. 1, Level 3, 3.1.4 List of studies to be generated, still ongoing or available but not evaluated, p.283	**Applicant**: Following concerns raised by the RMS in relation to the **impurity 6** content in the specification (please refer to the applicant comment no 5 for Vol. 4), the Applicant has initiated genotoxicity testing in accordance with the 2012 update of the technical equivalence guidance document (SANCO/10597/2003 –rev. 10.1). *(Applicant comment continues in next column -->)*	**The Applicant has initiated genotoxicity testing with proquinazid TGAI test material that contains a higher level of impurity 6**, that would be supportive of the specification. The **Ames (Study ID: 211857)** and **in vitro micronucleus (Study ID: 211856)** study reports will be available for the data call-in period. At this time, the draft data have indicated that the test material would not be considered genotoxic. Due to unexpected delays a **HPRT assay** has only recently been placed but the data and a summary will be available for the data call-in, with the final report to follow.

Genotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			The Applicant can also provide, upon request, a position paper updating the technical equivalence assessment in the context of the latest genotoxicity results and the new proposed specification content of **impurity 6** (0.9%). (Please refer to the applicant comment no 5 for Vol. 4). **Below listed new information (study reports, study summary and up-dated equivalency assessment) will be made available upon request.** • X11166670: Bacterial Reverse Mutation Test (Study ID: 211857) • X11166670: In Vitro Mammalian Cell Gene Forward Mutation Test at the HPRT Locus of the Chinese Hamster (CHO)-K1 Cell Line (Study ID: 230064) • X11166670: In Vitro Micronucleus Assay (Study ID: 211864) • Position paper updating the technical equivalence assessment.

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.6.5 Summary of long term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template] 2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target	**Applicant has conducted further mode of action work relevant to the carcinogenicity and endocrine assessments**. The report for the comparative liver enzyme induction assay is available upon request, with an associated position paper contextualising the findings. At which time the Applicant would like to suggest that the data be included in table 2.6.5-3.	The Applicant has conducted **a comparative liver enzyme study and comparative hepatocyte proliferation assay** (Study ID: 220243) in accordance with Appendix A of the ECHA/EFSA guidance document on the identification of endocrine disruptors, which supports the proposed mode of action for the liver-thyroid effects that have implications on the endocrine and carcinogenicity interpretation for this molecule. The Applicant considers that it can demonstrate a clear and marked quantitative difference between the levels of rat and human UGT-T4 activity

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	organ toxicity-repeated exposure), p.82-5 And, Table 2.6.5-3. Summary table of other studies relevant for long-term toxicity and carcinogenicity.	Furthermore, the Applicant would like the RMS to consider the inclusion of the 3 in vitro assays (thyroperoxidase (TPO), sodium iodide symporter (NIS) and thyroid receptor (TR) agonist assays) as these rule out alternate MOAs for the thyroid effect and support the proposed liver nuclear receptor-mediated MOA. Although the Applicant notes that these are included in the main text on p.101. *(Applicant comment continues in next column -->)*	after administration of proquinazid; Human hepatocytes require approximately 100 times higher concentration of proquinazid to see the same limited effect on UGT-T4 activity, above which concentration human hepatocytes start to show signs of cytotoxicity. With regards to the comparative hepatocyte proliferation assay no proliferative effects were seen in rat hepatocytes (consistent with 7d findings in the 2 yr carcinogenicity study), and no proliferation in human hepatocytes (positive controls in both species performed as expected). **These results when considered as a part of the endocrine and carcinogenicity assessment support the Applicant hypothesis of a rodent specific mode of action that is not relevant to humans.** **The study report (Study ID: 2220243) and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments will be provided upon request:** • In vitro CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the new comparative species in vitro hepatocyte enzyme and proliferation data in relation to the ED and carcinogenicity assessments.
(2)	Vol. 1, 2.6.5 Summary of long term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]	**Applicant** supports the position taken by the RMS with regards to the mode of action, and would like to make the RMS aware that the data suggested as a possible data gap will be available during the data call-in should the RMS/EFSA request the data.	A summary of the key findings can be found elsewhere in this commenting table, but the Applicant considers the study to meet the needs to the RMS and thus proposes no data gap would be required, if the study was requested by the authorities.

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		Furthermore, the Applicant would like to indicate that this study also addresses provides data pertaining to proliferation between rats and humans. *(Applicant comment continues in next column -->)*	***Please note the comparative liver enzyme data and proliferation data are in the same study report and will be provided upon request:*** • In vitro CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the new comparative species in vitro hepatocyte enzyme and proliferation data in relation to the ED and carcinogenicity assessments.
(3)	Vol. 1, 2.6.5 Summary of long term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template], p.103-4	**Applicant** supports the Carc. Cat. Proposed classification of proquinazid and would like to indicate that the **data available in the comparative liver enzyme assay (Study ID: 220043) which addresses the nonhuman relevance of the ED MOA and non-human relevance of the proliferative findings.** *(Applicant comment continues in next column -->)*	**This new report will be made available upon request by the RMS:** • In vitro CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the new comparative species in vitro hepatocyte enzyme and proliferation data in relation to the ED and carcinogenicity assessments.
(4)	Vol. 3, B.6.5.2 Carcinogenicity study in the mouse Vol. 1, Table 2.6.3.1-1. Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ	**Applicant** would like to reiterate their position on the presence of hepatocellular carcinomas in high-dose males for the RMSs reconsideration. *(Applicant comment continues in next column -->)*	As per the report, a slightly higher incidence of hepatocellular carcinomas was present in high dose males compared to controls. Incidences of hepatocellular carcinoma in male mice were 1/55, 0/55, 2/55, 0/56, 4/55 in the 0, 5, 30, 200, and 2000 ppm groups, respectively. This finding was considered not to be test substance-related based on the following: • The incidence of hepatocellular carcinoma in 2000 ppm males was not statistically increased by either the Cochran-Armitage trend test or the Fisher's exact test.

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	toxicity - repeated exposure). P.63-4 And, Vol. 1, Table 2.6.5-1. Summary table of animal studies on long-term toxicity and carcinogenicity.		• The 7.3% incidence in 2000 ppm males was equivalent to the upper bound of the laboratory historical control range of 0 – 6.3%. • Assessment of causality of hepatocellular tumours should include and assessment of adenomas and carcinomas combined (McConnell et al, 1986; Brix et al., 2010). No test substance-related increases hepatocellular adenomas, or combined hepatocellular tumours (adenoma plus carcinoma) were present in any treated group. In addition, there was no increase in foci of cellular alteration at any dose level.
(5)	Vol. 1, 2.6.5 Summary of long term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template], p.103-4	**Applicant:** On p.14, the RMS comments about a "Rodent specific mode of action for thyroid effects discussed in Volume 1" and the proposal for Carcinogenicity classification of Cat. 2. The Applicant would like to highlight that additional data is now available to confirm and elucidate the mode of action that is supportive of both endocrine disruption and carcinogenicity conclusions. *(Applicant comment continues in next column -->)*	The Applicant has conducted **a comparative liver enzyme study and comparative hepatocyte proliferation assay** (Study ID: 220043) in accordance with Appendix A of the ECHA/EFSA guidance document on the identification of endocrine disruptors, which supports the proposed mode of action for the liver-thyroid effects that have implications on the endocrine and carcinogenicity interpretation for this molecule. The Applicant considers that it can demonstrate a clear and marked quantitative difference between the levels of rat and human UGT-T4 activity after administration of proquinazid; Human hepatocytes require approximately 100 times higher concentration of proquinazid to see the same limited effect on UGT-T4 activity, above which concentration human hepatocytes start to show signs of cytotoxicity. With regards to the comparative hepatocyte proliferation assay no proliferative effects were seen in rat hepatocytes (consistent with 7d findings in the 2 yr carcinogenicity study), and no proliferation in human hepatocytes (positive controls in both species performed as expected).

Long-term toxicity and carcinogenicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			These results when considered as a part of the endocrine and carcinogenicity assessment support the Applicant hypothesis of a rodent specific mode of action that is not relevant to humans. **The study report (Study ID: 222043) and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments can be provided upon request.** • In vitro CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the new comparative species in vitro hepatocyte enzyme and proliferation data in relation to the ED and carcinogenicity assessments.

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, Table 2.6.6.1-1. Summary table of animal studies on adverse effects on sexual function and fertility – generational studies. p.106 And,	**Applicant:** In light of the comments made by the Applicant with regards to the B6 (Applicant comment 6 under Reproductive toxicity) pertaining to [redacted]-3080), the Applicant requests that the testicular histology deviation statement is removed from the table text p.106.	

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	Vol. 1, Table 2.6.3.1-1. Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure), p.65-6	The Applicant would like to refer the RMS to the histology reporting tables for the P1 (Table 80, p.169) and F1 (Table 82, p.177) males in the study report ([redacted]-3080) which includes the findings in the testes.	
(2)	Vol. 1, Table 2.6.6.2-1. Summary table of animal studies on adverse effects on development p.109 2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development, p.111 And, 2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development, p.112	**Applicant** respectfully requests that the RMS reconsider the inclusion of the RSO and PDA in light of the information provided in the 'further explanations' column of this row ([redacted]-1997-00707) in both the table text and the main body text of 2.6.6.2.1 and 2.6.6.2.2. Furthermore, that the RMS includes the body weight changes data which clearly demonstrates the presence of significant maternal toxicity. *(Applicant comment continues in next column -->)*	The Applicant would like to highlight the mean maternal body weight changes in table 2 of the report [redacted]-1997-00707 (p.31). There was a >50% reduction in the mean maternal body weight changes (minus the products of conception) over days 7 to 22 of gestation between controls (39.6g) and the 60 mg/kg bw/d groups (18.5g). There has clearly been significant maternal toxicity at this dose. The RSO incidences, whilst statistically significant, have a high natural background expression and these values are within the historical control data (as presented in the report and MCA5). Furthermore, the RSO and PDA are the final closing processes for soft and hard tissue and are findings common when there is a 10% reduction in foetal body weight. This with the lack of other supportive findings indicates that the RSO and PDA findings are likely driven by maternal toxicity. Therefore, the Applicant recommends the concluding statement by the RMS reflect both the presence of significant maternal toxicity and that the findings were within the background HCD if they wish to maintain a statement about these effects.

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			Regardless, the Applicant acknowledges and agrees with the RMS position that classification is not required.
(3)	Vol. 1, Table 2.6.6.2-1. Summary table of animal studies on adverse effects on development, p.109 And, 2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development, p.111	**Applicant** respectfully disagrees with the RMS including the increase in delayed pelvic ossification. The Applicant requests that the RMS revisit the data in light of the information provided by the Applicant in the 'Further explanations' column of this row and revise the table and main body text in 2.6.6.2.1 if in agreement. *(Applicant comment continues in next column -->)*	The Applicant would like to re-highlight that the incidences of delayed pelvic ossification are within the historical control data supplied in the study report and the MCA5. Furthermore, whilst the incidences have shown a statistical significance, the experimental unit of measure in these studies is the litter, not the incidences within the litter (Haseman, J.K. and Hogan, M.D. (1975). Selection of the Experimental Unit in Teratology Studies. Teratology, 12(2):165-171.). The incidence of this finding was 0 foetuses (0 litters), 0/0, 1/1, 4/2, and 6/2, from the 0, 1, 2.5, 5, and 10 mg/kg groups, respectively. Based on the experimental unit, the litter, this is essentially a flat response. Given this is also a non-adverse finding, the applicant would appreciate the reconsideration of the data and the provided reference by the RMS, and if in agreement a revision of their concluding statement to reflect the interpretation in light of the information provided here. Regardless, the Applicant acknowledges and agrees with the RMS position that classification is not required.
(4)	Vol. 3, CA B.6, B.6.6.1(02) Multigeneration reproduction study in rats	**Applicant** respectfully disagrees with the RMS with regards to the deviation mentioned in the bibliographic box "The RMS notes that testicular samples collected in this study were not histologically evaluated in accordance with the OECD 416 guideline (see below: Assessment and	The Applicant would like to refer the RMS to the histology reporting tables for the P1 (Table 80, p.169) and F1 (Table 82, p.177) males in the study report ([redacted]-3080) which includes the findings in the testes. The Applicant further requests that the RMS statements in the deviations section of the bibliographic box and the RMSs conclusions be edited accordingly.

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		conclusion by RMS)." (p.323) and in the RMS Conclusion (p.368-9). *(Applicant comment continues in next column -->)*	
(5)	Vol. 3, CA B.6, B.6.6.2(01) Teratogenicity test by the oral route in the rat,	**Applicant** respectfully disagrees with the RMSs concluding statement on p.379, the highlighted findings only occur in the clear presence of maternal toxicity. The Applicant requests that the RMSs statement more fully reflect this. *(Applicant comment continues in next column -->)*	The Applicant would like to highlight the mean maternal body weight changes in table 2 of the report [redacted]-1997-00707 (p.31). There was a >50% reduction in the mean maternal body weight changes (minus the products of conception) over days 7 to 22 of gestation between controls (39.6g) and the 60 mg/kg bw/d groups (18.5g). There has clearly been significant maternal toxicity at this dose. The RSO incidences, whilst statistically significant, have a high natural background expression and these values are within the historical control data (as presented in the report and MCA5). Furthermore, the RSO and PDA are the final closing processes for soft and hard tissue and are findings common when there is a 10% reduction in foetal body weight. This with the lack of other supportive findings indicates that the RSO and PDA findings are likely driven by maternal toxicity. Therefore, the Applicant recommends the concluding statement by the RMS reflect both the presence of significant maternal toxicity and that the findings were within the background HCD if they wish to maintain a statement about these effects. Regardless, the Applicant acknowledges and agrees with the RMS position that classification is not required.

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(6)	Vol. 3, CA B.6, B.6.6.2(02) Teratogenicity test by the oral route in the rabbit	**Applicant** respectfully disagrees with the RMSs concluding statement on p.387, that the increase in delayed pelvic ossification a statistically significant dose response. The Applicant requests that the RMSs revisit the data and revise the statement if in agreement. *(Applicant comment continues in next column -->)*	The Applicant would like to re-highlight that the incidences of delayed pelvic ossification are within the historical control data supplied in the study report and the MCA5. Furthermore, whilst the incidences have shown a statistical significance, the experimental unit of measure in these studies is the litter, not the incidences within the litter (Haseman, J.K. and Hogan, M.D. (1975). Selection of the Experimental Unit in Teratology Studies. Teratology, 12(2):165-171.). The incidence of this finding was 0 foetuses (0 litters), 0/0, 1/1, 4/2, and 6/2, from the 0, 1, 2.5, 5, and 10 mg/kg groups, respectively. Based on the experimental unit, the litter, this is essentially a flat response. Given this is also a non-adverse finding, the applicant would appreciate the reconsideration of the data and the provided reference by the RMS, and if in agreement a revision of their concluding statement to reflect the interpretation in light of the information provided here. Regardless, the Applicant acknowledges and agrees with the RMS position that classification is not required.
(7)	Vol. 1, Table 2.6.6.2-1. Summary table of animal studies on adverse effects on development, p.109-10.	**Applicant** respectfully suggest that the *Summary table of animal studies on adverse effects on development* be revised following the RMSs reconsideration of the data as pertaining to the Applicant's comments in points made in relation to Vol. 3, B.6.6.2(02) and Vol. 3, B.6.6.2(03). *(Applicant comment continues in next column -->)*	The Applicant considers that the inclusion of PDA and RSO for the rat, and delayed pelvic ossification for the rabbit, are not reflective of the findings of the study when maternal toxicity, HCD, type of effect and interpretive guidance are taken into account. The Applicant would like to express support that the findings of the reproductive and developmental toxicity studies submitted as part of the renewal do not constitute classification for reproduction in line with the previous assessment of the ECHA RAC.

Reproductive toxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(8)	Vol. 1, Table 2.9.1-2: Summary of toxicity of proquinazid to mammals, p.170	**Applicant** would like to highlight that the authors name and year for the reproductive toxicity two-generation study on p.170 should be corrected to Mylchreest, E., 2003. The study ID is correct.	

Neurotoxicity			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, Table 2.6.7-1. Summary table of animal studies on neurotoxicity.	**Applicant** recommends including the following statement from the B6 (p.406), which the RMS stated they agreed with, into the Vol. 1 *Table 2.6.7-1. Summary table of animal studies on neurotoxicity*: "On test day 15, there were no toxicologically relevant neurobehavioral effects in males or females at any dose".	

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.6.8.1, table 2.6.8.1 Mouse micronucleus test	**Applicant:** It is correct that this study was deficient in the number of immature erythrocytes scored for micronuclei (2000 instead of 4000). However, the MCA5 also identified a deviation in the number of total erythrocytes scored for immature erythrocyte	1000 erythrocytes were scored to determine the frequency of immature erythrocytes in the bone marrow smears. According to OECD TG474, 2000 erythrocytes should be scored where assessments are performed in peripheral blood. For this study, analysis was performed in bone marrow.

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		frequency. This second deviation was an error. OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored for bone marrow. In this study 1000 total erythrocytes were scored and therefore this study does not deviate from the TG with regards assessment of erythrocyte frequency. The applicant respectfully requests that the second part of this deviation is removed. *(Applicant comment continues in next column -->)*	
(2)	Vol. 1, 2.6.8.1, table 2.6.8.1 Mouse micronucleus test	**Applicant:** In the deviations column the third bullet point appears incomplete. However, under OECD TG 474 the requirement for concurrent plasma analysis is only necessary where there are no other data to demonstrate proof of exposure. As bone marrow toxicity was observed there is no requirement for plasma analysis. The applicant respectfully requests that the study is noted as accepted and reliable. *(Applicant comment continues in next column -->)*	Although no blood or plasma sample for potential analysis of proquinazid concentration was retained in this study this does not constitute a deviation as OECD TG474 recommends sample retention "where warranted and where other exposure data do not exist". As bone marrow exposure has been confirmed in this study (via the observations of target tissue toxicity), retention of a blood or plasma sample (together with any analysis of the sample) is not warranted. There is therefore no deviation from OECD TG 474.
(3)	Vol. 1, 2.6.8.1, Short summary. Study [redacted] 2201.	**Applicant** notes that the RMS has not entered the details of Vol. 3, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test into the table detailing studies on the IN-MM671 metabolite. The summary text	The applicant respectfully disagrees with the RMS's interpretation of "analysable" (as stated by OECD 471) and considers that at least 5 analysable concentrations were available in all experiments. For a plate to be analysable, it must be sufficiently free of confounding issues (such as excessive precipitate, discolouration of the agar or contamination) that it is possible to accurately

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		indicates that this is because the RMS did not accept the study. *(Applicant comment continues in next column -->)*	count the number of revertant colonies present and to assess the appearance of the background bacterial lawn. OECD 471 does not define "analysable" as being limited by a degree of cytotoxicity, indeed it is a requirement of OECD 471 for the test substance to be tested up to a cytotoxic concentration if cytotoxicity is occurring below 5 mg/plate or 5 µL/plate. In both experiments, both with and without metabolic activation, there are at least 5 analysable concentrations for all tester strains, where it was possible to accurately enumerate the revertant colonies and assess the appearance of the background bacterial lawn. This interpretation of "analysable" is supported by Levy et al (2019) Mutat Res Genet Toxicol Environ Mutagen. 848:403074. https://doi.org/10.1016/j.mrgentox.2019.07.004. The authors state "that there was no scientific basis for establishing a universal minimum number of non-toxic doses needed to establish the validity of a finding that a compound is non-mutagenic in the tests. Additional testing is not needed if a result is judged to be positive from an experiment with fewer than 5 analysable concentrations." The applicant considers the study to be reliable and acceptable.
(4)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P410	**Applicant:** Deviations from current test guideline. In error the MCA5 identified a lack of analysis on the formulated test material as a deviation from OECD 471 (1997). This is incorrect as neither the 1997 or 2020 versions of OECD 471 stipulate a requirement for analysis of the formulated test material. The applicant respectfully requests that this deviation is removed.	Analysis of formulated test material is a GLP requirement, however, it is not a requirement of OECD 471 and therefore there are no deviations from current test guideline for this study.

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		(Applicant comment continues in next column -->)	
(5)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P410	**Applicant:** The deviations attributed to the study author are not deviations from OECD TG471 and should be deleted. *(Applicant comment continues in next column -->)*	OECD TG 471 (2020) states that the following strains should be used: • S. typhimurium TA1537 **or** TA97 **or** TA97a • E. coli WP2 uvrA, **or** E. coli WP2 uvrA (pKM101), **or** S. typhimurium TA102. Therefore, the strains used are not deviations to TG471. OECD 471 provides examples of positive controls that could be used but does not stipulate that these must be used. Therefore, the use of ICR 191 is not a deviation from TG471.
(6)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P410	**Applicant:** Please change the vehicle from "None" to "dimethyl sulfoxide (DMSO).	
(8)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P411	**Applicant**: The text "and negative" should be deleted from the title of Table 6.8.1.002-1 as the table only details the Positive Controls. The negative (vehicle) control is documented with the test substance information	
(9)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P411	**Applicant:** Please present the E coli strain as WP2 uvrA (pKM101) in the bacterial strains column of Table 6.8.1.002-1.	

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		This will remove any confusion as to whether WP2 uvrA or WP2 uvrA (pKM101) was tested.	
(10)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P411	**Applicant:** The title of Table 6.8.1.002-2 is incorrect. Suggest that "Tester strains" may be a more appropriate title.	
(11)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P412-413	**Applicant:** In the results tables, please present the E coli strain as WP2 uvrA (pKM101). This will remove any confusion as to whether WP2 uvrA or WP2 uvrA (pKM101) was tested.	
(12)	Vol. 3, CA B.6, B.6.8.1(02), CA 6.8.1, Bacterial reverse mutation test. P412-413	**Applicant** respectfully disagrees with the RMS's interpretation of "analysable" (as stated by OECD 471) and considers that at least 5 analysable concentrations were available in all experiments. For a plate to be analysable, it must be sufficiently free of confounding issues (such as excessive precipitate, discolouration of the agar or contamination) that it is possible to accurately count the number of revertant colonies present and to assess the appearance of the background bacterial lawn. OECD 471 does not define "analysable" as being limited by a degree of cytotoxicity, indeed it is a requirement of OECD 471 for the test substance to be tested up to a cytotoxic concentration if cytotoxicity is occurring below 5 mg/plate or 5 µL/plate. In both experiments, both	This interpretation of "analysable" is supported by Levy et al (2019) Mutat Res Genet Toxicol Environ Mutagen. 848:403074. https://doi.org/10.1016/j.mrgentox.2019.07.004. The authors state "that there was no scientific basis for establishing a universal minimum number of non-toxic doses needed to establish the validity of a finding that a compound is non-mutagenic in the tests. Additional testing is not needed if a result is judged to be positive from an experiment with fewer than 5 analyzable concentrations." The applicant considers the study to be reliable and acceptable. In Experiment 2, for all strains tested with metabolic activation, there are revertant colonies present and a visible (albeit reduced in some instances) background bacterial lawn for at least 6 test substance concentrations. Without metabolic activation (minus S9), there are revertant colonies present and a visible (albeit reduced in some instances) background bacterial lawn for at least

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		with and without metabolic activation, there are at least 5 analysable concentrations for all tester strains, where it was possible to accurately enumerate the revertant colonies and assess the appearance of the background bacterial lawn. *(Applicant comment continues in next column -->)*	5 test substance concentrations for strains TA1535 and WP2 uvrA. For strain TA97a, without S9, although revertant colonies were present at only 4 concentrations, a visible and severely reduce background lawn was present at the 3 highest concentrations. Furthermore, in Experiment 1, there were revertant colonies and a visible (albeit reduced at the higher concentrations) background bacterial lawn for TA97a for all 7 concentrations tested. For strains TA98 and TA100, tested without S9, revertant colonies were present for only 4 test substance concentrations. However, in both experiments a visible (albeit reduced at high concentrations) background lawn was present for at least 5 test concentrations. Therefore, in all strains and in both experiments (with and without S9) there were at least 5 analysable concentrations, separated by less than half-log intervals. The study meets the exposure concentration requirements of OECD 471 and is valid and reliable for mutagenicity evaluation. IN-MM671 is non-mutagenic in the Bacterial Reverse Mutation Assay using Salmonella typhimurium and Escherichia coli.
(13)	Vol. 3, CA B.6, B.6.8.1(03), CA 6.8.1, Mouse bone marrow micronucleus test. P414	**Applicant**: It is correct that this study was deficient in the number of immature erythrocytes scored for micronuclei (2000 instead of 4000). However, the MCA5 also identified a deviation in the number of total erythrocytes scored for immature erythrocyte frequency. This second deviation was an error. OECD TG 474 (2016) recommends at least 500 total erythrocytes are scored for bone marrow. In this study 1000 total erythrocytes were scored and therefore this study does not deviate from the TG with regards assessment of erythrocyte frequency. *(Applicant comment continues in next column -->)*	The applicant respectfully requests that the second part of this deviation is removed. 1000 erythrocytes were scored to determine the frequency of immature erythrocytes in the bone marrow smears. According to OECD TG474, 2000 erythrocytes should be scored where assessments are performed in peripheral blood. For this study, analysis was performed in bone marrow.

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(14)	Vol. 3, CA B.6, B.6.8.1(03), CA 6.8.1, Mouse bone marrow micronucleus test. P414	**Applicant:** Under OECD TG 474 the requirement for concurrent plasma analysis is only necessary where there are no other data to demonstrate proof of exposure. As bone marrow toxicity was observed there is no requirement for plasma analysis. The applicant respectfully requests that the study is noted as accepted and reliable. *(Applicant comment continues in next column -->)*	Although no blood or plasma sample for potential analysis of proquinazid concentration was retained in this study this does not constitute a deviation as OECD TG474 recommends sample retention "where warranted and where other exposure data do not exist". As bone marrow exposure has been confirmed in this study (via the observations of target tissue toxicity), retention of a blood or plasma sample (together with any analysis of the sample) is not warranted. There is therefore no deviation from OECD TG 474.
(15)	Vol. 1, 2.10.2.1 ED assessment for T-modality, p.195 2.10.2.1.2 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality, p.216 (2nd row of the table) 2.10.2.1.3.2 Further information to be generated to postulate MoA, p.218	**Applicant:** The Applicant would like to highlight that a comparative enzyme induction assay in hepatocytes has been conducted and the report will be available upon request, removing the need for a data gap. *(Applicant comment continues in next column -->)*	The Applicant has conducted comparative species (rat and human) liver enzyme study and comparative hepatocyte proliferation assay (Study ID: 220243) which supports the proposed mode of action for the liver-thyroid effects that have implications on the endocrine and carcinogenicity interpretation for this molecule. The Applicant considers that it can demonstrate a clear quantitative difference between the levels of rat and human UGT-T4 activity after administration of proquinazid; Human hepatocytes require around 100 times higher concentration of proquinazid to see the same limited effect on UGT-T4 activity, after which concentration human hepatocytes start to show signs of cytotoxicity. With regards to the comparative hepatocyte proliferation assay no proliferative effects were seen in rat hepatocytes (consistent with 7d findings in the 2 yr carcinogenicity study), and no proliferation in human hepatocytes (positive controls in both species performed as expected).

Further toxicological studies			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	2.10.2.1.4 Conclusion on the assessment of T-modality, p.221 2.10.2.3 Overall conclusion on the ED assessment for humans 2.10.4 Overall conclusion on the ED assessment And, Vol. 1, Level 3, 3.1.4 List of studies to be generated, still ongoing or available but not evaluated, p.283		These results when considered as a part of the endocrine and carcinogenicity assessment support a rodent specific **mode of action that is not relevant to humans.** **The study report (Study ID: 220243) and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments will be provided upon request:** • In vitro CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the impact of the new data on the CLH carcinogenicity assessments and endocrine conclusion

Toxicological end points: ADI, ARfD, AOEL			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, Level 3, 3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of	**Applicant**: With regards to the endocrine conclusion and carcinogenicity classification, the Applicant supports the RMSs proposal of 'does not meet the criteria' for an endocrine disruptor and Carc. Cat. 2.	The Applicant has conducted a comparative liver enzyme study and comparative hepatocyte proliferation assay (report ID: 220243) which supports the proposed mode of action for the liver-thyroid effects that have implications on the endocrine and carcinogenicity interpretation for this molecule.

Toxicological end points: ADI, ARfD, AOEL			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	Regulation (EC) No 1107/2009, p.277-8 Vol. 1, Level 3, 3.1.8 Area(s) where expert consultation is considered necessary, p.287 And, Vol. 1, Level 3, 3.2 Proposed decision, p.288	The Applicant would also like to highlight that a comparative enzyme induction assay in hepatocytes has been conducted and the report will be available upon request, removing the need for a data gap. *(Applicant comment continues in next column -->)*	The Applicant considers that it can demonstrate a clear quantitative difference between the levels of rat and human UGT-T4 activity after administration of proquinazid; Human hepatocytes require around 100 times higher concentration of proquinazid to see the same limited effect on UGT-T4 activity, after which concentration human hepatocytes start to show signs of cytotoxicity. With regards to the comparative hepatocyte proliferation assay no proliferative effects were seen in rat hepatocytes (consistent with 7d findings in the 2 yr carcinogenicity study), and no proliferation in human hepatocytes (positive controls in both species performed as expected). These results when considered as a part of the endocrine and carcinogenicity assessment support the Applicant hypothesis of a rodent specific mode of action that is not relevant to humans. **The study report (Study ID: 2220243) and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments will be provided upon request:** • In vitro CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the impact of the new data on the CLH carcinogenicity assessments and endocrine conclusion

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals [equivalent to section 9 of the CLH report template] p.35	**Applicant** agrees with the RMS conclusion noting that "The toxicokinetic profile of proquinazid **have been reliably and acceptably studied, and there is nothing to indicate it would influence classification**. "	
(2)	Vol.1 2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template] p.35-36	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification for acute oral toxicity** according to Regulation (EC) No. 1272/2008.	
(3)	Vol. 1, 2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template] p.37	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification for acute dermal toxicity** according to Regulation (EC) No. 1272/2008.	
(4)	Vol. 1, 2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template] p.38	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification for acute inhalation toxicity** according to Regulation (EC) No. 1272/2008.	
(5)	Vol. 1, 2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template] p.39-40	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification for skin corrosion/irritation** according to Regulation (EC) No. 1272/2008.	

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(6)	Vol. 1, 2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template] p.40-41	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification for serious eye damage/eye irritation** according to Regulation (EC) No. 1272/2008.	
(7)	Vol. 1, 2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template] p.41-42	**Applicant** agrees with the RMS conclusion for **non-relevance**.	
(8)	Vol 1, 2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template] p.43	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification** for according to Regulation (EC) No. 1272/2008.	
(9)	Vol. 1, 2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template] p.45	**Applicant** agrees with the RMS conclusion for proquinazid **does not fulfil criteria for classification for aspiration hazard** according to Regulation (EC) No. 1272/2008.	
(10)	Vol.1, 2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template] p.46-51	**Applicant** agrees with the RMS conclusion for proquinazid **does not warrant classification as Specific Target Organ Toxicity – Single Exposure** according to Regulation (EC) No. 1272/2008.	

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(11)	Vol.1, 2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report] Vol. 1, 2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure) p. 52-85	**Applicant** agrees with the RMS conclusion for proquinazid **does not meet the criteria for classification as STOT RE** according to Regulation (EC) No. 1272/2008; and **the MoA of adverse thyroid effects in rats is considered rodent specific, and therefore not relevant to humans.** *(Applicant comment continues in next column -->)*	In further support of this position the **Applicant has generated additional information (Study ID: 220243) on the mode of action and human relevance which is relevant to the CLH assessment for carcinogenicity.** The additional data is also relevant to the endocrine conclusion, as the data supports the proposed liver-thyroid mode of action and demonstrates non-human relevance. Study report is due to finalisation (*end March 2023*) **The study report and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments will be provided upon request:** • *In vitro* CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the impact of the new data on the CLH carcinogenicity assessments and endocrine conclusion Briefly, the Applicant has conducted comparative species (rat and human) liver enzyme and hepatocyte cell proliferation studies. Whilst there is no defined test guideline for these assays, they were conducted based on published procedures, and the liver enzyme assays are in line with the ECHA-EFSA ED guidance document Appendix A. The results of the comparative liver enzyme study demonstrate a clear quantitative difference between the differential (delta) UGT-T4 activity levels of rat and human hepatocytes after administration of proquinazid. Human hepatocytes require approximately 100 times higher concentration of proquinazid to see the same limited effect on UGT-T4 activity as observed in the rat; concentration above that limited effect level produce cytotoxicity in human hepatocytes. Given that the follicular changes in the rat thyroid are likely driven by T4-UGT induction (with resulting increased TH turnover) and

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			the clear difference between rats and humans with regards to UGT-T4 induction, the thyroid changes observed in rats are likely not relevant to humans at plausible exposure scenarios and thus support the non-human relevance for the thyroid tumours observed in rats. With regards to the comparative hepatocyte proliferation assay (report ID: 220243), no proliferative effects were seen in rat hepatocytes (consistent with 7d findings in the 2yr rat carcinogenicity study), and no proliferation in human hepatocytes (positive controls in both species performed as expected). The Applicant considers this important to the CLH classification assessment with regards to the liver tumours, indicating these have non-human relevance. **Overall, these results when considered as a part of the endocrine and carcinogenicity assessment support a rodent specific mode of action that is not relevant to humans**
(12)	Vol.1, 2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template] p. 85-91	**The Applicant** is in support of the RMS proposal for **no genotoxicity/mutagenicity classification required**, which is in alignment with the current ECHA harmonised classification. All available data confirms that **no genotoxicity concerns for proquinazid.** For detailed comments on the genotoxicity and generated new data please refer to the applicant comments provided under the genotoxicity section.	

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(13)	Vol. 1, 2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template], p.91-104	**Applicant** is in support of the **RMS proposal for Carc. Cat 2. Classification,** which is in alignment with the current ECHA harmonised classification. In further support of this position the **Applicant has generated additional information (Study ID: 220243) on the mode of action and human relevance which is relevant to the CLH assessment for carcinogenicity.** The additional data is also relevant to the endocrine conclusion, as the data supports the proposed liver-thyroid mode of action and demonstrates non-human relevance. Study report is due to finalisation (*end March 2023*) *(Applicant comment continues in next column -->)*	**The study report and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments will be provided upon request:** • *In vitro* CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243) • Position paper addressing the impact of the new data on the CLH carcinogenicity assessments and endocrine conclusion Briefly, the Applicant has conducted comparative species (rat and human) liver enzyme and hepatocyte cell proliferation studies. Whilst there is no defined test guideline for these assays, they were conducted based on published procedures, and the liver enzyme assays are in line with the ECHA-EFSA ED guidance document Appendix A. The results of the comparative liver enzyme study demonstrate a clear quantitative difference between the differential (delta) UGT-T4 activity levels of rat and human hepatocytes after administration of proquinazid. Human hepatocytes require approximately 100 times higher concentration of proquinazid to see the same limited effect on UGT-T4 activity as observed in the rat; concentration above that limited effect level produce cytotoxicity in human hepatocytes. Given that the follicular changes in the rat thyroid are likely driven by T4-UGT induction (with resulting increased TH turnover) and the clear difference between rats and humans with regards to UGT-T4 induction, the thyroid changes observed in rats are likely not relevant to humans at plausible exposure scenarios and thus support the non-human relevance for the thyroid tumours observed in rats. With regards to the comparative hepatocyte proliferation assay (report ID: 220243), no proliferative effects were seen in rat hepatocytes (consistent

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			with 7d findings in the 2yr rat carcinogenicity study), and no proliferation in human hepatocytes (positive controls in both species performed as expected). The Applicant considers this important to the CLH classification assessment with regards to the liver tumours, indicating these have non-human relevance. **Overall, these results when considered as a part of the endocrine and carcinogenicity assessment support a rodent specific mode of action that is not relevant to humans.** For further comments on the carcinogenicity please refer to the applicant comments provided under the Long-term toxicity and carcinogenicity section.
(14)	Vol. 1, 2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template] 2.6.6.1 Adverse effects on sexual function and fertility 2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template] 2.6.6.3 Adverse effects on or via lactation [equivalent to section	**Applicant** is in support of the RMS proposal for **no reproductive toxicity classification** required, which is in alignment with the current ECHA harmonised classification. All available data confirms that **no reproductive toxicity concerns for proquinazid.** For further comments on the reproductive toxicity please refer to the applicant comments provided under the reproductive toxicity section.	

Other comments, proposals for classification			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	10.10.7 of the CLH report template] p. 104-113		

List of New Information that Applicant would like to provide

1) **The study report and a position paper addressing the impact of the new data on the endocrine and carcinogenicity assessments:**

   - *In vitro* CYP and UGT induction and cell proliferation in Sprague-Dawley rat and human hepatocytes by X11166670 (Study ID: 220243)
   - Position paper addressing the impact of the new data on the CLH carcinogenicity assessment and endocrine conclusion

2) **New Genotoxicity testing with proquinazid TGAI test material that contains a higher level of impurity-6:**

   - X11166670: Bacterial Reverse Mutation Test (Study ID: 211857)
   - X11166670: In Vitro Mammalian Cell Gene Forward Mutation Test at the HPRT Locus of the Chinese Hamster (CHO)-K1 Cell Line (Study ID: 230064)
   - X11166670: In Vitro Micronucleus Assay (Study ID: 211864)
   - Position paper updating the technical equivalence assessment.

Further Comments of Applicant (Corteva Agriscience) on the CLH report on Proquinazid

and Annexes: CLH_REP_ATT_SPS-023780-22_Proquinazid_RAR_10_Volume_3CA_B-8_2022-09-19 & CLH_REP_ATT_SPS-023780-22_Proquinazid_RAR_11_Volume_3CA_B-9_2022-09-19

02.02.2023

Complimentary to the comments provided via online commenting form, applicant, Corteva Agriscience would like to provide further and more detailed comments to address specific datapoints of and discussions provided in the **CLH Report** & **its annexes** *(correspondence to: Vol 1 and Vol 3 of the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008)).*

Provided comments are in parallel (but selected to *relevance*) with those submitted under Call for comments on the Renewal Assessment Report of Proquinazid (AIR IV) announced by EFSA (EFSA-Q-2018-00520).

Below provided comments also includes the new information generated by applicant and considered "important" for the evaluation and decision making of RAC. Aforementioned new information (study reports, position papers) will be provided to ECHA / RAC review upon request.

CONTENT

A) Environmental fate and behaviour

- **Route and rate of degredation in soil**
- **Fate and behaviour in water and sediment and effect of water treatment procedures on the nature of residues**
- **Fate and behaviour in water and sediment and effect of water treatment procedures on the nature of residues**
- **Fate and behaviour in air**

B) Ecotoxicology

- **Aquatic organisms**

C) List of new Information that Applicant would like to provide

A. Environmental fate and behaviour

Route and rate of degradation in soil			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(13)	Vol.1, 2.8.1.3 Assessment in relation to the P-criteria	**Applicant:** On page 156 it is stated that "In conclusion thus, taken both laboratory and field data into consideration, **proquinazid is not considered to fulfil the criteria as P."** **Applicant agree with the not Persistent classification**.	

Fate and behaviour in water and sediment and effect of water treatment procedures on the nature of residues			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(3)	Vol. 1, Level 2 **2.8.2.2.8 Assessment of P-criteria for water and sediment** Page 168 And Vol 1, Level 3	**Applicant:** On page 168 RMS stating that *"In conclusion, given the importance of the aerobic mineralisation tests together with the findings in the water/sediment tests (geomean DT50 total system of 68.2 days), the RMS considers that proquinazid fulfils the POP/vPvB and PBT criteria."* *(Applicant comment continues in next column -->)*	Applicant would like to note that, **a new irradiated water-sediment** study has been conducted (Study ID: 220042) to assess the degradation of proquinazid under sunlight conditions, as photolysis is expected to be a significant route of degradation. The results of this study have shown that the rate of degradation in water and sediment is significantly reduced under sunlight and conclusively demonstrated that proquinazid is not persistent in aquatic environments. Initial findings have shown that the rate of degradation in water and sediment is significantly reduced under sunlight with DT50 in sediment approximate of 5 days and total system of <1 day. **Aforementioned new study will be made avalible upon request**.

Further Comments of Applicant (Corteva Agriscience) on the CLH report on Proquinazid

Annex: CLH_REP_ATT_SPS-023780-22_Proquinazid_RAR_10_Volume_3CA_B-8_2022-09-19

Annex: CLH_REP_ATT_SPS-023780-22_Proquinazid_RAR_11_Volume_3CA_B-9_2022-09-19

Fate and behaviour in water and sediment and effect of water treatment procedures on the nature of residues			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	3.1.2 Proposal - Candidate for substitution Page 281		• McLaughlin, 2023, "[14C]Proquinazid - Aerobic Transformation in Aquatic Sediment Systems in the presence of Sunlight" (Study ID: 220042) **The study results clearly demonstrates that proquinazid does not fulfils the P criteria of POP/vPvB and PBT classifications*. Respectively no* CfS classification required.**

Fate and behaviour in air			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
(1)	Vol. 1, 2.8.3.1.2 Comparison with the CLP criteria Page 169	**Applicant agrees with the RMS conclusion that** "Since proquinazid is non-volatile and not included in Annex I or Annex II to II to Regulation (EC) 1005/2009 it can be concluded that it **does not fulfil classification criteria for 'hazardous to the ozone layer'**.	

B. Ecotoxicology

Aquatic organisms			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
5	Vol 3 CA / B.9.2.2.2 Fish full life cycle test / CA 8.2.2 And Vol 1 3.1.4.9 Level 3 List of studies to be generated, still ongoing or available but not evaluated And Vol 1 3.1.5 Issues that could not be finalised point 3	**Applicant:** On page 90 it is stated that *"The RMS agrees that the trigger value related to BCF (>1000) is not fulfilled. However, the persistence trigger (total water-sediment system DegT90 >100 days) was significantly breached, with whole system DegT90 of 436 and 118 days in the two test systems, respectively. From our point of view, not only the water phase should be considered for the trigger of a FFLC study, but also persistence in sediment. It is not clear whether the rapid photolysis in water would be considered sufficient to demonstrate that the substance is rapidly degraded under natural conditions. Hence, further data to address the chronic toxicity to fish should be considered."* *(Applicant comment continues in next column -->)*	Applicant believes that the **FFLC study is not triggered on the basis of the available data set, and Guideline as BCF is below <1000.** Nevertheless, to address the concerns of the RMS on the persistence, applicant carried out a **new irradiated water-sediment study** OECD 308 to assess the degradation of proquinazid under sunlight conditions, as photolysis is an expected route of degradation. Initial findings have shown that the rate of degradation in water and sediment is significantly reduced under sunlight with DT50 in sediment approximate of 5 days and total system of <1 day, which is expected as based on the known photolytic degradation of proquinazid. **This study will be made available upon request:** • McLaughlin, 2023, "[14C]Proquinazid - Aerobic Transformation in Aquatic Sediment Systems in the presence of Sunlight" (Study ID: 220042) This new study clearly indicates that proquinazid is much less persistent in water/sediment under more realistic conditions indicated by the irradiated water-sediment study. **More detailed position paper on this and the overall aspect that a <u>FFLC is not triggered</u> can be provided by the APPL upon request.** Due to the significant role played by photolysis in the overall degradation of proquinazid from the environment the applicant has conducted this additional study to assess the fate and behaviour of proquinazid in aquatic compartments under more realistic conditions in the presence of light. The

Aquatic organisms			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
			results of this study indicate that degradation of proquinazid from the aquatic environment occurs more rapidly in the presence of light resulting in much shorter half-lives in both water and sediment compartments than those obtained from a study conducted exclusively in the dark. Accordingly, it can be conclusively shown that light plays a key role in the degradation/dissipation of proquinazid from the aquatic environment and based on these findings proquinazid is not persistent in water or sediment. Additionally, **In conclusion, Corteva Agriscience believes a fish full lifecycle study for proquinazid is not warranted for the following reasons:** • **Bioaccumulation potential is less than the trigger of 1000 (Study ID: [redacted]-8197)** • **Fish chronic toxicity has been robustly assessed in two fish early-stage studies and a fish short term reproduction study (Study ID: [redacted]-1998-00638, Study ID: [redacted] 4486-97 and Study ID: 190011)** • **Exposure is limited based on known photolytic and hydrolytic degradation of the active**
6	Vol. 3, B.9.2.3, Potential for endocrine disruption, KCA 8.2.3/01 p.90-95	**Applicant:** Response to RMS on MTC setting. In addition to mortality, the 96-hour range finding test indicated that significant morbidity (40%) was observed at 261 µg/L. Consequently, the next lowest treatment of 172 µg/L was selected as the MTC. This was chosen as the range finder was only 4-days compared to the 21-day definitive assay. Therefore, it was considered important to avoid morbidity in the definitive test.	Applicant appreciates and acknowledges RMS conclusion on acceptability of the study. Treatments that exhibited overt toxicity would have had no value in the determination of potential endocrine activity. Consequently, the MTC was selected to avoid these effects in the definitive test. It is also important to note that the high-test level of 176.3 µg/L exceeds the LOECs for mortality from the fish early lifestage studies (58 µg/L in rainbow trout and 18.9 µg/L in sheepshead minnow).

Aquatic organisms			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		Morbidity is considered a sign of overt toxicity that would have compromised the treatment level (see paragraph 69, page 20; OECD TG231). *(Applicant comment continues in next column -->)*	Applicant believes the MTC was sufficient for the purposes of the assay.
7	Vol. 3, B.9.2.3, Potential for endocrine disruption, KCA 8.2.3/01 Vol 1 Page 253 2.10.3.3 Overall conclusion on the ED assessment for non-target organisms and 2.10.4 Overall conclusion on the ED assessment	**Applicant:** Response to RMS comments on nHLL. As indicated the APPL has submitted an amended report and position paper to the RMS, which has not been evaluated by RMS yet as acknowledge. This amendment corrects the statistical analysis by accounting for late-stage animals. This statistical analysis, as required by the Test Guideline, indicates no statistically significant effect on nHLL at any treatment level or time interval. *(Applicant comment continues in next column -->)*	None of the conditions outlined in the TG's decision logic (see Figure 3; paragraph 46; OECD TG231) indicating thyroid activity were met (including accelerated development as evidenced by a lack of increase in nHLL and lack of changes in median developmental stage/developmental stage distribution). **Thus, it can be concluded that proquinazid is considered thyroid inactive in the Amphibian Metamorphosis Assay.** **Amended study report and the position paper can be provided again upon request.** • [redacted], 2013, Proquinazid (DPX-KQ926) technical: 21-D amphibian metamorphosis assay (AMA) with South African clawed frog, Xenopus laevis, (Study ID: [redacted]36329), **Revision 1(December 2021)** • Position Paper: Applicants Comments on the RMS concerns relevant to Amphibian Metamorphosis Assay for Proquinazid and Re-evaluation of endpoints related to T-modality in non-target organisms
8	Vol. 3, B.9.2.3, Potential for endocrine disruption, KCA 8.2.3/02	**Applicant:** Response to RMS comments on VTG levels in female fish. Statistical analysis with and without outliers removed is included in the study report. However, this is not clearly presented in the study	

Aquatic organisms			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
		summary included in the dossier (except in the Applicant comment box). **Updated study summary will be provided upon request.** Updated will be provided to more clearly indicate that the statistically significant increase in female VTG is no longer statistically significant when outliers are removed.	
9	Vol. 3, B.9.2.3, Potential for endocrine disruption, KCA 8.2.3/02	**Applicant:** Response to RMS comments on slight oocyte atresia observed in females at 28 and 88 µg a.s./L treatment groups. As commented previously oocyte atresia is a common background finding that may also occur in response to a variety of non-specific stressors. Further, the response did not follow a concentration response relationship. **No evidence of EAS-mediated endocrine activity since slight oocyte atresia is not a specific histopathology of EAS-mediated endocrine activity and no other treatment-related gonad histopathology was observed. On request the APPL can provide historical control** data that demonstrates the levels of atresia observed are within the historical control of fathead minnow Fish Short Term Reproduction Assays.	

Aquatic organisms			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
21	Vol 3 B.9.2.5 Long-term and chronic toxicity to aquatic invertebrates CA 8.2.5.1	**Applicant** acknowledges and agrees with the RMS that the Daphnia study (Kreamer, 1998) is acceptable. On the other hand, in recognition of the deviations listed for the study, to address current guideline requirements **applicant carried out a new Daphnia study (Study ID: 211087),** *which confirms the current endpoints.* *(Applicant comment continues in next column -->)*	***The new Daphnia study will be made available upon request.*** • Gerke et al.,2021, PROQUINAZID: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*) (Study ID: 211087)
22	Vol 3 B.9.2.5 Long-term and chronic toxicity to aquatic invertebrates **New Study**	**Applicant** conducted a **new study with Lumbriculus** to assess the potential toxicity in sediment. *(Applicant comment continues in next column -->)*	***This study will be made available upon request.*** • Billa et al., 2022, PROQUINAZID: A PROLONGED SEDIMENT TOXICITY TEST WITH Lumbriculus variegatus USING SPIKED SEDIMENT (Study ID: 211160)
	Vol.1. 2.9.2.1. Bioaccumulation [equivalent to section 11.4 of the CLH report template] **2.9.2.2. Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]**	**Applicant:** On page 178 it is noted that *"Based on the available data on bioaccumulation summarized above, the BCF trigger (500) for chronic aquatic hazard according to CLP is fulfilled. Regarding criteria for PBT and vPvB, the triggers (2000 and 5000, respectively) are not met."* On page 182 it is noted that *"The acute toxicity of proquinazid to both fish (LC50 = 0.349 mg/L), aquatic invertebrates (EC50 = 0.110 mg/L) and algae (no effect up to 0.12 mg/L (mean measured)) fulfils*	**Applicant agrees with the RMS conclusion for the Acute aquatic hazard evaluation and proposed classification.** On the other hand, applicant acknowledges that, based on available experimental data, a steady state, whole fish wet weight lipid normalised and growth corrected BCF value of 813 was derived for proquinazid. This exceeds the BCF criteria of 500 for bioaccumulation according to CLP. However, **A new irradiated water-sediment study has** been conducted (Corteva study 220042, McLaughlin, 2023) to assess the degradation of proquinazid under sunlight conditions, as photolysis is an expected route of

Aquatic organisms			
No.	Column 1 Reference to assessment report	Column 2 Comment (restricted to 500 characters, ca.10 lines)	Column 3 Further explanations
	2.9.2.3. Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template] **2.9.2.4.1. Acute aquatic hazard** **2.9.2.5. Conclusion on classification and labelling for environmental hazards** **2.9.2.4.2. Long-term aquatic hazard (including bioaccumulation potential and degradation)** **environmental hazards**	*the classification criterion of ≤ 1 mg/L for Category Acute 1 according to Regulation (EG) 1272/2008.* *The proposed M-factor is 1 (appropriate for acute toxicity values within in the range 0.1 – 1.0 mg/L)."* *(Applicant comment continues in next column -->)*	degradation. Initial findings have shown that the rate of degradation in water and sediment is significantly reduced under sunlight with DT50 in sediment approximate of 5 days and total system of <1 day. These new available rate of degradation may also have an impact on BCF values, as overall exposure may be significantly reduced then under realistic conditions. Final study report including confirmed values will be provided to add more information to the classification of Proquinazid in water and sediment as well as long term aquatic hazard classification and bioaccumulation classification. Applicant also generated 2 new aquatic organisms' studies which provides additional information (confirms and/or improves the current end points) for the classification evaluation for aquatic hazard. In this scope, applicant would like to present these 3 new study to for the consideration of RAC and RMS/EFSA upon request: • Mclaughlin, 2023, Irradiated Water-Sediment Study (Study ID: 220042) • Billa et al., 2022, PROQUINAZID: A PROLONGED SEDIMENT TOXICITY TEST WITH Lumbriculus variegatus USING SPIKED SEDIMENT (Study ID: 211160) • Gerke et al.,2021, PROQUINAZID: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*) (Study ID: 211087)

Further Comments of Applicant (Corteva Agriscience) on the CLH report on Proquinazid

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List of New Information that Applicant would like to provide

1) Mclaughlin, 2023, Irradiated Water-Sediment Study (Study ID: 220042)
2) Gerke et al.,2021, PROQUINAZID: A SEMI-STATIC LIFE-CYCLE TOXICITY TEST WITH THE CLADOCERAN (*Daphnia magna*) (Study ID: 211087)
3) Billa et al., 2022, PROQUINAZID: A PROLONGED SEDIMENT TOXICITY TEST WITH Lumbriculus variegatus USING SPIKED SEDIMENT (Study ID: 211160)
4) [redacted] 2013, Proquinazid (DPX-KQ926) technical: 21-D amphibian metamorphosis assay (AMA) with South African clawed frog, Xenopus laevis, (Study ID: [redacted]36329), **Revision 1(December 2021)**
5) Position Paper: Applicants Comments on the RMS concerns relevant to Amphibian Metamorphosis Assay for Proquinazid and Re-evaluation of endpoints related to T-modality in non-target organisms