Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

pyraclostrobin (ISO); methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate

EC Number: -
CAS Number: 175013-18-0

CLH-O-0000007219-70-01/F

Adopted

1 December 2022

1 December 2022

CLH-O-0000007219-70-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **pyraclostrobin (ISO); methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate**

EC Number: **-**

CAS Number: **175013-18-0**

The proposal was submitted by **Germany** and received by RAC on **20 September 2021.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **29 November 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **11 February 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ifthekhar Ali Mohammed**

Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **1 December 2022** by **consensus**.

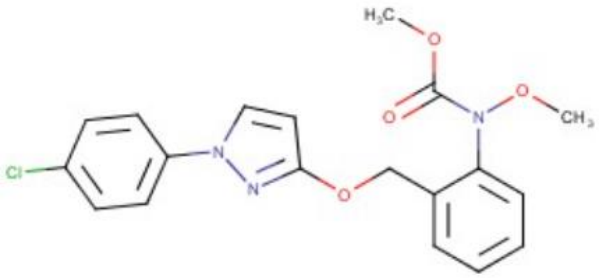
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-272-00-6	pyraclostrobin (ISO); methyl *N*-{2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl]phenyl}(N-methoxy)carbamate	-	175013-18-0	Acute Tox. 3* Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H331 H315 H400 H410	GHS06 GHS09 Dgr	H331 H315 H410		M=100	
Dossier submitters proposal	613-272-00-6	pyraclostrobin (ISO); methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate	-	175013-18-0	**Retain** Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2 Acute Tox. 4 STOT SE 3 STOT RE 2 **Modify** Acute Tox. 3	**Retain** H331 H315 H400 H410 **Add** H361d H302 H335 H373 (liver, gastrointestinal tract)	**Retain** GHS06 GHS09 Dgr **Add** GHS08	**Retain** H331 H315 H410 **Add** H361d H302 H335 H373 (liver, gastrointestinal tract)		inhalation: ATE = 0.58 mg/L (dusts or mists) oral: ATE = 450 mg/kg bw M = 100 M = 100	
RAC opinion	613-272-00-6	pyraclostrobin (ISO); methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate	-	175013-18-0	**Retain** Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 **Add** Repr. 2 Acute Tox. 4 STOT SE 3 STOT RE 2 **Modify** Acute Tox. 3	**Retain** H331 H315 H400 H410 **Add** H361d H302 H335 H373 (liver, gastrointestinal tract, nasal cavity)	**Retain** GHS06 GHS09 Dgr **Add** GHS08	**Retain** H331 H315 H410 **Add** H361d H302 H335 H373 (liver, gastrointestinal tract, nasal cavity)		inhalation: ATE = 0.58 mg/L (dusts or mists) oral: ATE = 450 mg/kg bw M = 100 M = 100	
Resulting Annex VI entry if agreed by COM	613-272-00-6	pyraclostrobin (ISO); methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate	-	175013-18-0	Repr. 2 Acute Tox. 3 Acute Tox. 4 Skin Irrit. 2 STOT SE 3 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H331 H302 H315 H335 H373 (liver, gastrointestinal tract, nasal cavity) H400 H410	GHS08 GHS06 GHS09 Dgr	H361d H331 H302 H315 H335 H373 (liver, gastrointestinal tract, nasal cavity) H410		inhalation: ATE = 0.58 mg/L (dusts or mists) oral: ATE = 450 mg/kg bw M = 100 M = 100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Pyraclostrobin (ISO) is a fungicidal active substance used in plant protection products.



Structure of pyraclostrobin

Pyraclostrobin is a white or light beige solid at room temperature with a melting point range of 63.7 °C – 65.2 °C, a water solubility of 1.9 ± 0.17 mg/L (at 20 °C in deionized water, pH of 5.8) and a log K_{ow} of 3.99.

Toxicokinetics

Three *in vivo* studies via oral administration (an OECD test guideline TG 417 study each in Wistar rats and NMRI mouse and a metabolism study in Wistar rats according to the method EEC 87/302) and a comparative *in vitro* metabolism study are reported in the CLH report for pyraclostrobin.

After a single dose of 5 or 50 mg ^{14}C-radiolabeled pyraclostrobin/kg bw to Wistar rats, oral absorption was found to be rapid but incomplete. At 120 h post-dosing, only 15 % of the applied radioactivity was excreted via the urine, whereas elimination via the faeces accounted for 80-90 % of the dose. With the biliary excretion of approx. 35% determined at 48 h post-dosing, an oral bioavailability of 50% is assumed for both sexes. There was no evidence of accumulation of pyraclostrobin in this study and the highest amount of radioactivity was found in the gastro-intestinal tract followed by the liver. All other tissues had values comparable to or less than the plasma concentrations (TOX2000-705: 1998).

After a single oral dose of 300 mg radiolabelled pyraclostrobin/kg bw to NMRI mouse, radioactivity was detectable after 2 h in the systemic circulation, in the bone marrow and in the liver (ASB2017-5506: 2016).

The systemically available portion of pyraclostrobin was rapidly and extensively metabolised in Wistar rats. N-desmethoxylation was the quantitatively most important pathway. No major differences were observed with regard to sex and dose level (TOX2000-708: 1999).

In a comparative *in vitro* metabolism study in hepatocytes from humans, rabbits, rats and dogs, many similarities but also remarkable differences were noted for pyraclostrobin. At the highest concentration of 10 µM, the cytotoxicity in human cells was much greater compared to that in rats and rabbits. However, there was no difference noted at the lower concentration of 3 µM. Metabolism of pyraclostrobin appeared faster in cells and microsome preparations obtained from rabbits and rats as compared to those from humans and it was slowest in dog microsomes. Qualitatively, the rabbit metabolite profile was most similar to that of humans. A few metabolites that were abundant in human and rabbit cells were not identified in rat samples (ASB2015-8295: 2014).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Pyraclostrobin is an active substance in plant protection products that is not currently listed in Annex VI of Regulation (EC) No 1272/2008 for physical hazards. The Dossier Submitter (DS) proposed no classification for all physical hazards. The substance is solid which means that hazard classes related to gases and liquids are not relevant for its physical hazard classification.

Explosives

Pyraclostrobin was tested for explosive properties using EC Method A.14 and was found not to be explosive. However, the method EC A.14 is not sufficient on its own to conclude on explosive properties. The differential scanning calorimetry (DCS) result shows an energy release over 1200 J/g. Pyraclostrobin contains functional groups associated with explosive properties (Table A6.1 in Appendix 6 of the UN RTDG) and the calculated oxygen balance is not less than -200. Therefore, it cannot be excluded that the substance might have explosive properties.

According to the CLP Regulation (Annex I, 2.1.2.3.), explosive properties are tested using UN test series 2 to 8. Corresponding UN test results were not available.

The DS concluded that data are not sufficient for concluding on classification.

Flammable solids

Three studies on flammability performed according to EC A.10 test showed that pyraclostrobin is not highly flammable. Substance did not burn under test conditions. Since the available data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary (ECHA Guidance, Chapter R.7a: Endpoint specific guidance, R.7.1.10.3). As a conclusion, the DS proposed no classification of pyraclostrobin.

Self-reactive substances

Pyraclostrobin contains functional groups which are associated with explosive properties (Table A6.1 in Appendix 6 of UN RTDG) and functional groups indicating self-reactive properties (Table A6.3 in Annex 6 of UN RTDG).

According to CLP Regulation, self-reactive properties are tested using UN test series A to H; the hazard class can be assessed also based on the criteria in CLP Annex I, 2.8.4.2 (waiver). Since no corresponding UN test results are available and the substance contains above mentioned groups, it cannot be excluded that the substance has self-reactive properties.

DS concluded that data are not sufficient for concluding on classification.

Pyrophosphoric solids

No studies are available. Based on practical experience in handling the substance, pyraclostrobin does not ignite spontaneously on coming into contact with air at normal temperatures. No classification was proposed by the DS.

Self-heating substances

Two studies conducted in accordance with EC A. 16 are available. In these studies, pyraclostrobin did not self-ignite up to 400°C. Thus, classification as a self-heating for the substance does not apply. However, the results from this study are not conclusive to assess this hazard class.

According to CLP Regulation, self-heating properties are tested using UN test N.4. No corresponding UN test results are available.

Therefore, DS concluded that data are not sufficient for classification.

Substances which in contact with water emit flammable gases

The chemical structure of the substance does not contain metals or metalloids and, based on experience in handling showing that the substance does not react with water. As a conclusion, the DS proposed no classification of pyraclostrobin.

Oxidising solids

Provided studies performed according to test method EC A.17 indicated that pyraclostrobin is not considered an oxidising substance. However, results from method EC A.17 are not sufficient to conclude on oxidising properties. Since no UN test O.1 or O.3 was conducted, the available data are not sufficient for the assessment of the oxidising properties (CLP Regulation, Annex I, 2.14.2.1.). Therefore, DS concluded that data are not sufficient for classification.

Organic peroxides

Hazard class not applicable for pyraclostrobin as the substance does not contain the peroxide group (-O-O-).

Corrosive to metals

Hazard class not applicable as only solids with a melting point below 55 °C need to be tested. Pyraclostrobin does not fulfill this criterion.

Desensitised explosives

Due to lack of data the hazard class was not assessed by DS but was open for consultation.

Comments received during consultation

Member State Competent Authorities (MSCA) and a company-manufacturer provided comments.

In the view of the MS a DSC (differential scanning calorimetry) measurement should be performed to confirm the absence of classification of pyraclostrobin as self-reactive substance.

Regarding self-heating substances, the MS was of the opinion that the justification "*as the melting point of the substance is below 160°C, no further test is needed for classification according to CLP guidance*" should be added.

The Company-Manufacturer provided the results of the new studies for some hazard classes and their position regarding the proposed classification.

- Explosives: The new study (BASF DocID 2020/2027396) using required UN test series indicated that substance is not considered to exhibit a danger of explosion. The provided data (method used and results) are presented in the section Additional key elements. The results are not leading to a classification according to GHS and transport classification class 1.
- Self-reactive substances: In the new study (BASF DocID 2020/2027397) using UN test H.4 the SADT (self-accelerated decomposition temperature) was determined to be higher than 75°C. According to CLP Annex I, 2.8.4.2, the classification procedures for self-reactive substances and mixtures need not to be applied if the SADT is greater than 75°C. Consequently, pyraclostrobin does not need to be classified as a self-reactive substance.

- Self-heating substances: The new study (BASF DocID 2020/2027396) reports the following results determined in a Grewer oven screening test: The sample showed a self-heating at 103°C (47°C). But the wire basket was empty after the test and the melting point is appr. 70°C, which is below 160°C. Therefore, the UN N.4 test was omitted. Pyraclostrobin does not need to be classified as self-heating substance.
- Oxidising solids: In the new study (BASF DocID 2020/2027396) using UN test method O.3 the mean burning rate of the sample-mixtures is less than the mean burning rate of the reference mixture. Due to these test results, pyraclostrobin does not need to be classified as oxidizing solid according to GHS and transport classification class 5.1.
- Desensitised explosives: This hazard class is not relevant, because pyraclostrobin is not required to be classified as an explosive.

Assessment and comparison with the classification criteria

During the preparation of the first draft opinion, new studies mentioned in the public consultation by the Company-Manufacturer were provided (BASF DocID 2020/2027396 and 2020/2027397). All the tests were carried out based on the methods referred to in Part 2 of Annex I to CLP (UN RTGD, Manual of Tests and Criteria). The study (BASF DocID 2020/2027396) is also in compliance with GLP.

RAC is of the opinion that it is appropriate to consider data from these new studies for classification of the substance as they were indicated during the Consultation, were received in sufficient time to be fully evaluated and were conducted according to appropriate methods.

Explosives

Pyraclostrobin is not considered to be explosive based on Method EC A.14. However, the results from this study are not conclusive as the method is not totally in line with the CLP Regulation. The negative A.14 test provides supporting information that pyraclostrobin is not explosive; however, it is not sufficient to conclude on the classification.

Screening procedure (Annex I, section 2.1.4.3 of the CLP Regulation)

Based on the screening procedure applied to pyraclostrobin, the substance does not fulfil any of the conditions set out in CLP Regulation, Annex I, 2.1.4.3, (a-c). Therefore, the substance could be a potential explosive and thus the acceptance procedure has to be performed (CLP Guidance, section 2.1.4.2.).

- Pyraclostrobin has chemical groups which may indicate explosive properties according to Table A6.1 in Appendix 6 of the UN RTDG. Two contiguous nitrogen atoms are present in the pyrazole ring and N-O group.
- Pyraclostrobin contains groups associated with explosive properties which include oxygen and calculated oxygen balance is not less than -200.
- Pyraclostrobin was found to have a measured exothermic decomposition energy of 1490 J/g which is higher than the indicated value of 500 J/g in Table A6.2 of UN MTC and the decomposition onset temperature of the substance is 145°C which is lower than indicated temperature of 500°C in Table A6.2 of UN MTC.

Acceptance procedure (Annex I, Figure 2.1.2 of the CLP Regulation)

Since pyraclostrobin is neither manufactured with a view to using it for practical explosive purposes or pyrotechnic effects, nor is it a candidate for ammonium nitrate emulsion, suspension or gel, the first question refers to the basic explosive properties investigated in Test series

number 1. This test line-up answers the question "*Is it an explosive substance/mixture?*" based on three assays:

- Type 1 (a): a shock test with defined booster and confinement to determine the ability of the substance to propagate a detonation (*UN Gap test*, zero gap);
- Type 1 (b): a test to determine the effect of heating under confinement (Koenen test);
- Type 1 (c): a test to determine the effect of ignition under confinement (Time/pressure test).

Pyraclostrobin was not subjected to type 1(a) test (UN Gap test, zero gap*); however, according to the paragraph 2.1.4.2 of CLP Regulation, if the exothermic decomposition energy of organic materials is 800 J/g or more, tests 1 (a) and 2 (a) need not be performed if the outcome of the ballistic mortar Mk.IIId test (F.1), or the ballistic mortar test (F.2) or the BAM Trauzl test (F.3) with initiation by a standard No 8 detonator (Appendix 1 to the UN RTDG, Manual of Tests and Criteria) is 'no'. In this case, the results of test 1 (a) and 2 (a) are deemed to be '-'.* Since pyraclostrobin has an exothermic decomposition energy of 1490 J/g and result of the BAM Trauzl test (F.3) is 'no', waiving this test was appropriate.

Pyraclostrobin was positive in a Koenen test and negative in a time/pressure test. *According to paragraph 2.1.4.5.1 of CLP guidance, the question is answered 'Yes' if a '+' is obtained in any of the three types of tests.* As the Koenen test has shown a positive conclusion the line of decision has to be continued.

The next question that has to be answered is the following: "Is the substance/mixture too insensitive for acceptance into this Class?" The response is given following the results obtained in Test Series 2. This battery of assays comprises the same tests as the Series 1 but with less stringent criteria.

- Type 2 (a): a shock test with defined initiation system and confinement to determine sensitivity to shock (UN gap test) (with a defined gap e.g., 50 mm);
- Type 2 (b): a test to determine the effect of heating under confinement (Koenen test);
- Type 2 (c): a test to determine the effect of ignition under confinement (Time/pressure test).

The UN gap test is waived for pyraclostrobin as shown before. Pyraclostrobin was negative in a Koenen test and time/pressure test. *According to paragraph 2.1.4.5.1 of CLP guidance, if the answer is 'Yes', the substance is rejected from this class; it is not an explosive.*

Overall, based on Test Series 1 and Test Series 2 of the decision logic the pyraclostrobin does not meet the classification criteria for classification as explosive.

According to the CLP Guidance (paragraph 2.1.4.5.1), it is recommended to carry out Test Series 3 before Test Series 1 and 2 for safety reasons due to the small sample amount needed. It is also recommended to carry out Test Series 3 even if negative results have been obtained in Test Series 1 and/or 2 because only Test Series 3 gives information about the thermal stability and the sensitivity to mechanical stimuli (impact and friction). Test Series 3 is used to answer the questions 'Is the substance/mixture thermally stable?' and 'Is the substance/mixture too dangerous for transport in the form in which it was tested?' This involves tests for determining the sensitiveness of the substance or mixture to mechanical stimuli (impact and friction), and to heat and flame. The following four types of tests are used (recommended test is indicated within brackets):

- Type 3 (a): a falling weight test to determine sensitiveness to impact (BAM Fallhammer);
- Type 3 (b): a friction; or impacted friction test to determine sensitiveness to friction (BAM friction apparatus);
- Type 3 (c): an elevated temperature test to determine thermal stability (thermal stability test at 75 °C);

- Type 3 (d): an ignition test to determine the response of a substance or mixture to fire (small scale burning test).

Based on results from preliminary tests on mechanical sensitivity, impact sensitivity (3(a)) and friction sensitivity (3(b)), pyraclostrobin successfully passed the Type 3(a) and Type 3(b) tests. The results for Type 3 (c) and Type 3 (d) tests are not available.

In conclusion, RAC is of the opinion that based on applied acceptance procedure (CLP Regulation, Annex I, Figure 2.1.2) and supported by negative EU A.14 test **no classification for explosives is warranted for pyraclostrobin**.

Flammable solids

Pyraclostrobin was tested for flammability using UN test N.1 which has demonstrated that the substance is not flammable. The results of the experimental test do not fulfil the criteria in CLP Regulation, Table 2.7.1.

Pyraclostrobin was not considered to be highly flammable in an experimental study performed according to method EU A.10. RAC notes that the result "not highly flammable" complies with the ECHA guidance on information requirements and chemical safety assessment (R.7.1.10.3), wherein it is stated that data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary.

Overall, RAC is of the opinion that **no classification for flammable solids is warranted for pyraclostrobin**.

Self-reactive substances

Based on the screening procedure (CLP Regulation, Annex I, 2.8.4.2) applied to pyraclostrobin, the substance has chemical groups which may indicate explosive properties according to Table A6.1 in Appendix 6 of the UN RTDG. Two contiguous nitrogen atoms are present in the pyrazole ring and N-O group. In addition, the substance also has chemical groups associated with self-reactive properties according to Table A6.3 in Appendix 6 of the UN RTDG. Two phenyl groups are present (unsaturation, olefins).

Pyraclostrobin was tested for self-reactive substance using UN test H.4 which has shown that SADT [self-accelerating decomposition temperature] was higher than 75°C. Result of this study support no classification of pyraclostrobin as a self-reactive substance as the substance fulfills the condition set out in CLP Regulation, Annex I, 2.8.4.2, b.

RAC is of the opinion that **no classification for self-reactive substance is warranted for pyraclostrobin**.

Pyrophoric solids

According to classification considerations in CLP Regulation Annex I, 2.10.4.1., the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperature. The substance is known to be stable at room temperature for prolonged periods of time (days). Thus, RAC agrees with the DS proposal **not to classify pyraclostrobin as a pyrophoric solid**.

Self-heating substances

EC A.16 tests showed no self-ignition below the melting point. However, a study conducted in accordance with EC A.16 is not sufficient to conclude on the classification according to CLP guidance (2.11.4.2.). The negative A.16 test provides supporting information that pyraclostrobin is not self-heating substance.

The screening procedure applied to pyraclostrobin has shown that the substance fulfils the condition set out in CLP guidance, section 2.11.4.2.:

- Pyraclostrobin has a melting point in the range 63.7 °C – 65.2 °C)/appr. 70°C (submitted study) which is below the 160 °C.
- Pyraclostrobin was completely molten at 160°C (data from submitted study).

Results from the Grewer oven screening test indicated that the sample showed self-heating at 103°C but the wire basket was empty after the test. The substance was molten at appr. 70°C.

Pyraclostrobin was not subjected to UN Test N.4; however, *according to the paragraph 2.11.4.4.1 of CLP guidance (version 5, July 2017), the test procedure need not be applied if the substance or mixture is completely molten at 160 °C.* Since pyraclostrobin has the melting temperature in the range 63.7 °C – 65.2 °C (CLP report)/appr. 70°C (submitted study) which is considerably lower than 160 °C, waiving this test was appropriate. Waiving of the test was supported also by one commenting MS and DS in the public consultation.

RAC is of the opinion that **no classification for self-heating substances is warranted** for pyraclostrobin based on screening procedure (2.11.4.2., CLP guidance (melting point)) and supported by a negative A.16 test.

Substances which in contact with water emit flammable gases

Based on the screening procedure applied to pyraclostrobin, the substance fulfills all criteria as set out in CLP Regulation, Annex I, 2.12.4.1, a-c. Consequently, the test can be waived (CLP guidance, 2.12.4.2.).

- Pyraclostrobin does not contain metals or metalloid.
- Experience in production and handling shows that the substance does not react with water.
- Pyraclostrobin is soluble in water (1.9±0.17 mg/L).

RAC agrees with the DS **not to classify pyraclostrobin as a substance which in contact with water emit flammable gases.**

Oxidising solids

Screening procedure (Annex I, section 2.14.4.1 of the CLP Regulation)

Based on the screening procedure applied to pyraclostrobin, the substance does not fulfil any of the conditions set out in the CLP regulation, Annex I, 2.14.4.1, a-b. Therefore, the substance could be regarded as potentially oxidising and thus further testing is required (CLP guidance, section 2.14.4.1.1.).

- Pyraclostrobin contains oxygen and chlorine.
- Pyraclostrobin contains oxygen and chlorine, and the oxygen is chemically bonded to other (nitrogen) than carbon or hydrogen.

<u>Testing requirements (CLP guidance, section 2.14.4.3.)</u>

According to CLP guidance, 2.14.4.1.1 if the substance is 'potentially oxidising' the further testing is required. It is not possible to assign a hazard category on the basis of a theoretical evaluation (based on composition and chemical structure).

The result from the UN MTC Test O.3 (Gravimetric test for oxidizing solids) has shown test has shown that pyraclostrobin is not an oxidizing solid, as the mean burning rate of the sample-mixtures was less than the mean burning rate of the reference mixture. The result of this study supports no classification of pyraclostrobin as an oxidizing solid as the conditions set out in Table 2.14.1 of the CLP regulation are not fulfilled.

Pyraclostrobin had no oxidizing properties according to EC A.17., however this test is not considered in the CLP criteria for this purpose and thus is not sufficient to conclude that the substance is not oxidising (results can be regarded as inconclusive). However, it provides supporting information that pyraclostrobin is not oxidizing solid.

RAC is of the opinion that **no classification for oxidising solids is warranted** for pyraclostrobin based the results of UN O.3 test and supported by negative EC A.17 test.

Organic peroxides

RAC agrees with DS that this hazard class is not applicable to pyraclostrobin as the substance does not contain the peroxide group (-O-O-).

Corrosive to metals

RAC agrees with the DS **not to classify pyraclostrobin as corrosive**. According to the CLP Guidance, section 2.16.4.1. only solids having a melting point lower than 55 °C (test temperature required in UN Test C.1) must be taken into consideration. No corrosiveness to metals is expected for pyraclostrobin as its melting point is 63.7 °C – 65.2 °C which is above 55 °C.

Desensitised explosive

Pyraclostrobin fulfils the condition set out in CLP Regulation, Annex I, 2.17.4.1, (a), thus the classification procedure for desensitised explosives does not apply.

- Pyraclostrobin contains no explosives according to the criteria in CLP Regulation, Annex I, Section 2.1.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

No mortality was observed in an acute oral toxicity study (OECD TG 401, vehicle: aqueous tylose) in Wistar rats for pyraclostrobin up to 5000 mg/kg bw. However, the dossier submitter (DS) proposed Acute Tox. 4; H302 for pyraclostrobin for acute oral toxicity based on an LD_{50} of approx. 450 mg/kg bw (the ATE) calculated in a dose-range finding study (vehicle: olive oil) for *in vivo* micronucleus test in NMRI mice.

Acute dermal toxicity

No mortality was observed in an acute dermal toxicity study (OECD TG 402) in Wistar rats for pyraclostrobin up to 2000 mg/kg bw. No other relevant studies were identified by the DS. Therefore, the DS proposed "no classification" for pyraclostrobin for acute dermal toxicity.

Acute inhalation toxicity

In two acute inhalation toxicity studies (OECD TG 403) in Wistar rats for pyraclostrobin (98% purity, liquid aerosol), LC_{50}-values of 0.69 mg/L and 0.58 mg/L, respectively, were obtained. In a third acute inhalation toxicity study (according to OECD TG 403) in Wistar rats for pyraclostrobin (only 40% of the active substance in the test material, liquid aerosol), the

calculated LC_{50}-values (for 100% active substance) were >1.55 & <2.78 mg/L for males and 2.1 mg/L for females.

The DS proposed Acute Tox. 3; H331 with an ATE of 0.58 mg/L (dusts or mists) for pyraclostrobin for acute inhalation toxicity.

Comments received during consultation

Three MSCAs and a Company/Manufacturer submitted comments during the consultation. The three MSCAs supported the DS proposal. The Company/manufacturer commented that the higher acute oral toxicity of the lipophilic pyraclostrobin in mice is likely caused by the non-aqueous vehicle (olive oil) enhancing the adsorption in the gastro-intestinal tract. The DS responded that their proposal is appropriate in accordance with the Guidance on the Application of the CLP Criteria (CLP guidance, ECHA, 2017) which states that the classification is based on the lowest ATE available taking into consideration the different species (rat vs mouse) or vehicles used (aqueous tylose vs olive oil).

Assessment and comparison with the classification criteria

Acute oral toxicity

In an acute oral toxicity study (OECD TG 401, GLP-compliant and no deviations) with pyraclostrobin (purity: 98.5%, vehicle: aqueous tylose) in Wistar rats (5/sex/dose), a single oral dose of 2000 or 5000 mg/kg bw resulted in no mortality (observation period: 15-d post dosing). Clinical signs such as dyspnoea, apathy, staggering, piloerection and diarrhoea were noted in both sexes at the low and high dose levels, but they disappeared within a few days after administration (TOX2000-709: 1998).

In a dose-range finding (DRF) study for an *in vivo* micronucleus test in NMRI mice (males (M) and females (F)), pyraclostrobin (purity: 98.2%, vehicle: olive oil) was given at different single oral doses ranging from 125 to 2000 mg/kg bw (observation period: 5 days post dosing). A combined LD_{50} of approx. 450 mg/kg bw was calculated for both sexes. Clinical signs (piloerection and hunched posture) were seen at dose levels of 250 mg/kg bw and higher. At higher doses (≥ 400 mg/kg bw), further clinical signs (e.g., reduced general state, salutatory spasm, irregular respiration) were reported (ASB2017-5505: 2016). The mortality data from this DRF study is reported in the table below.

***Table**: Mortality data from the DRF study (ASB2017-5505: 2016) for in vivo micronucleus test in mice (Table 17 in the CLH report)*

Dose level [mg/kg bw]	Animals treated (M/F)	Dead animals (M/F; time of death)
2000	2/2	2/2 (within 15 min)
1000	4/4	4/4 (15 min to 1 d)
500	4/4	3/3 (30 min to 2 d)
400	5/5	1/1 (1 d)
300	5/5	0/0
250	5/5	0/0
125	5/5	0/0

In the DRF study for *in vivo* micronucleus test in mice, 3 out of 4 animals tested (for each sex) died within 30 minutes to 2 days at 500 mg/kg bw while 1 of 5 animals tested (for each sex) died within 1 day at 400 mg/kg bw. RAC notes that the observation period post-dosing is shorter

(5 days) in this study, which raises an uncertainty as to whether a longer, standard (14 days) observation period possibly could have led to higher mortality and thus a lower LD_{50}. Although the DRF study is not a standard acute oral toxicity study, RAC considers the calculated LD_{50} of approx. 450 mg/kg bw for both sexes in this study as appropriate to base the acute oral toxicity classification on. Considering the lipophilic nature of pyraclostrobin (log P_{ow} of 3.99), olive oil is an appropriate vehicle.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Acute Tox. 4; H302 with an ATE of 450 mg/kg bw.**

Acute dermal toxicity

In an acute dermal toxicity study (according to OECD TG 402, GLP-compliant and no deviations) with pyraclostrobin (purity: 98.2%, dissolved in a 0.5% aqueous (bidest) Tylose® CB 30,000, semi-occlusive application) in Wistar rats (5/sex), a single exposure for 24 hours of 2000 mg/kg bw resulted in no mortality (observation period: 14 days post application). One day after application, very slight to well-defined erythema, a mechanical skin lesion due to the adhesive nature of the test substance was observed in all animals. No pathological findings were detected in the animals at necropsy (TOX2000-710: 1998). RAC notes that the aqueous vehicle used may not have completely dissolved the lipophilic pyraclostrobin.

Since there were no mortalities at 2000 mg/kg bw in the standard study, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for acute dermal toxicity.**

Acute inhalation toxicity

In the first acute inhalation toxicity study (OECD TG 403, GLP-compliant and no significant deviations affecting study validity) with pyraclostrobin (purity: 98.2%, liquid aerosol, mass median aerodynamic diameter (MMAD) between 1.0 and 2.9 µm with a geometric standard deviation (GSD) between 2.5 and 3), Wistar rats (5/sex/group) were exposed nose-only for 4 hours to 0, 0.31, 1.07 or 5.3 mg/L (observation period: 14 days post exposure). There was 100 % mortality at the two highest concentrations within 75 minutes of exposure. Necropsy of the mid-concentration (1.07 mg/L) animals showed agonal congestive hyperaemia. There was no mortality at the lowest concentration (0.31 mg/L) but clinical signs such as irregular respiration, bloody nose discharge, piloerection and smeared fur were observed until day 7 post-exposure. The calculated LC_{50} in this study was 0.69 mg/L (TOX2000-711: 1997).

In the second acute inhalation toxicity study (OECD TG 403, GLP-compliant and no deviations) with pyraclostrobin (purity: 98.2%, liquid aerosol, MMAD between 1.2 and 1.7 µm with GSD between 2.5 and 2.7), Wistar rats (5/sex/group) were exposed nose-only for 4 hours to 0, 0.52, 0.65 or 0.85 mg/L (observation period: 14 days post-exposure). There was 100% mortality at the highest concentration (0.85 mg/L), 90% mortality at the mid-concentration (0.65 mg/L) and 10% mortality at the lowest concentration (0.52 mg/L). All deaths occurred on the day of exposure. Gross necropsy of the animals that died during the study revealed mainly red discolorations of the lungs. In most cases, all lung lobes were affected. Additionally, wet and contaminated fur was observed in animals at the mid-concentration level that died during the study. One mid-concentration male also displayed lung oedema in all lobes. Clinical signs were observed at all concentrations and consisted of visually accelerated respiration, attempts to escape, squatting posture and piloerection, but these signs had resolved by day 7 of the observation period at the latest. The calculated LC_{50} in this study was 0.58 mg/L (ASB2008-5020: 2002).

In the third acute inhalation toxicity study (OECD TG 403, GLP-compliant and no deviation) with pyraclostrobin (formulation containing only 38.1% active substance, liquid aerosol, MMAD between 2.7 and 4.3 µm with GSD between 2.5 and 2.7), Wistar rats (5/sex/group) were exposed

nose-only for 4 hours to 0, 0.89, 1.96, 4.07 or 7.3 mg/L (observation period: 14 days post exposure). There was 90% mortality at the highest concentration. Few deaths (10%) and clinical signs were noted at the two mid-concentrations. The calculated LC_{50}-values (for 100% active substance) in this study were >1.55 & <2.78 mg/L for males and 2.1 mg/L for females (TOX2001-881: 2001).

The LC_{50}-values from the first two studies (0.69 and 0.58 mg/L) are in the range for Acute toxicity Category 3 ($0.5 < LC_{50} \leq 1.0$ mg/L (dusts/mists)). The LC_{50}-values in the third study are above the limit for Category 3; however, this study was conducted with a formulation and a higher upper range of MMAD compared to the other two studies. Overall, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Acute Tox. 3; H331 with an ATE of 0.58 mg/L (dusts or mists).**

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

Summary of the Dossier Submitter's proposal

No significant target organ findings were reported in the acute toxicity studies with pyraclostrobin except in the lungs in the acute inhalation toxicity studies at lethal doses. The DS proposed classification as STOT SE 3 for respiratory tract irritation based on human poisoning cases after exposure to pyraclostrobin products and supported (in the RCOM) by respiratory irritation seen in animal inhalation studies (at non-lethal doses).

Comments received during consultation

One MSCA commented and did not support the DS proposal. The MSCA pointed to the lack of details in the human poisoning cases, including the composition of the products. Additionally, they considered that there is no evidence of respiratory tract irritation in the animal data.

In response, the DS acknowledged that evaluation of human data is often hindered by limitations in reporting of exposure conditions. The DS emphasised the effects relevant for classification in the human and the animal data and also pointed out that in the renewal assessment report (RAR) the applicant self-classified pyraclostrobin as STOT SE 3 for respiratory tract irritation.

Assessment and comparison with the classification criteria

Human data

The applicant provided information from the clinical incident log at the occupational site where workers were exposed to pyraclostrobin products in combination with other active ingredient containing products. Some cases of slight irritation of the eyes, skin and mouth and/or intoxication (indisposition, headache, ague, fatigue, aching muscles, vomiting, drowsiness, dizziness, adynamic feet, breathing difficulties) were reported. No details of these case reports are available to the DS or RAC.

In a publication by Gergely *et al*. (2007), five independent events of low to moderate severity poisoning by pyraclostrobin products were reported which all occurred in the US state of Iowa, with a total of 33 persons affected. The patients complained about irritation and pain of the upper respiratory tract as well as nausea, headache, eye pain, weakness, dizziness, and chest pain.

In the first event, when working among crops, 27 migrant workers (20 men and 7 women) were accidentally exposed to pyraclostrobin product that was sprayed from a crop-duster plane. Twenty-six of them complained of upper respiratory tract pain or irritation; 20 of chest pain; 3 of nausea, and 1 patient each had pruritis, skin redness, eye pain, weakness, headache, or exhibited dizziness, respectively. During the RAC-63 meeting, the Industry representative confirmed that the exposure in this event was primarily to pyraclostrobin as it was identified from the samples taken from safety glasses of the workers.

There were three other events of exposure to pyraclostrobin products due to off-target drift from nearby aerial applications. In total, 5 people were affected. They were exposed when riding a motorcycle near a field that was just under treatment or by spray drifting to their home yard. Headache and eye pain partially associated with conjunctivitis and dizziness were reported. No respiratory irritant effects were reported.

In the fifth reported event, a crop-duster pilot was dermally exposed to the spilled liquid pyraclostrobin product when his plane crashed during take-off. The pilot exhibited chemical burns but reported no respiratory symptoms.

Animal data

In the acute inhalation toxicity study (TOX2000-711: 1997), in the low concentration group (0.31 mg/L) with no mortality, 6/10 rats had irregular respiration only during the 4-h exposure and in 2 male rats bloody nose discharge was seen within 1 day after exposure.

In another acute inhalation toxicity study (ASB2008-5020: 2002), visually accelerated respiration was observed in all groups including in the low concentration group (0.52 mg/L) with 10% mortality.

RAC notes that the effects on respiration observed in the animal studies occur at dose levels that are (close to) being lethal but support that the respiratory system is a target organ.

Overall, RAC considers that the first event reported in the Gergely *et al*. (2007) publication with pyraclostrobin products is sufficient for classification. Thus, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as STOT SE 3; H335: May cause respiratory irritation.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an acute dermal irritation/corrosion study (OECD TG 404) in rabbits, none of the 6 animals tested had a mean score of ≥2.3 and ≤4.0 for erythema/eschar or for oedema. However, the DS proposed Skin Irrit. 2 for pyraclostrobin based on skin irritation that was not reversible within 15 days in 2 animals in the OECD TG 404 study and the irritation observed in the skin sensitisation study (OECD TG 406, Guinea pig maximisation test - GPMT) in a weight of evidence approach.

Comments received during consultation

Three MSCAs and a Company/manufacturer submitted comments during the consultation. Two MSCAs supported the DS proposal. One MSCA did not support the proposal noting that in the RAR for pyraclostrobin, it is stated that remaining test material was seen at termination in the two animals in the OECD TG 404 study, and they considered that this most likely induced mechanical irritation. This MSCA and the company, which also did not support the DS proposal,

cautioned on the use of irritation data from the GPMT due to differences in exposure of the substance compared to OECD TG 404 and the use of an adjuvant. The company also commented that according to the CLP guidance (2017), classification for irritation is applicable if at least 4 out of 6 rabbits show a mean score of ≥2.3 and ≤4.0 for erythema/eschar or for oedema. The company considered that the same numerical criteria (4 of 6 animals) should apply to the non-reversibility of skin irritation at the end of the observation period.

In response, the DS noted that in the OECD TG 404 study, remaining test material was found on all rabbits until 8 days post application, but some rabbits recovered by 72 hours even though the test material was remaining. They also noted that the study authors explicitly reported any mechanical irritation observed and that this was only seen in one rabbit up to 24 hours post application. Thus, the DS considered that the irritation findings are most likely due to the chemical nature of the test material. The DS also mentioned that the authors of the OECD TG 404 study concluded that pyraclostrobin was a skin irritant.

The DS noted that the CLP guidance (2017) does not specify any numerical threshold of animals for non-reversibility of skin lesions for studies with more than 3 animals and did not agree with the company. In support of this the DS cited the CLP guidance (2017). According to section 3.2.2.3.2.2 of the CLP guidance (2017), the irritation criteria for classification are fulfilled if

- "a limited degree of alopecia, hyperkeratosis, hyperplasia and scaling occurs. Two animals showing this response are sufficient for the classification as irritant."
- "very elevated mean scores throughout the study are revealed, including lesions persisting at the end of an observation period of normally 14 days. One animal showing this response throughout and at the end of observation period is sufficient for the classification as irritant".

The DS acknowledged that the experimental design of the GPMT is not directly comparable to the OECD TG 404 study and that intradermal injection of an adjuvant can trigger irritating effects. However, the DS noted that in the pre-test of the GPMT the maximum non-irritant concentration of pyraclostrobin was 1% in the two 24-h percutaneous occlusive applications implying that pyraclostrobin might be a potent skin irritant and exposure to higher concentration for a shorter period (e.g., 4 hours) could also lead to skin irritation. The DS considered this information as supporting evidence for classification purposes.

Assessment and comparison with the classification criteria

In the acute dermal irritation/corrosion study (OECD TG 404, GLP-compliant and no deviations), 0.5 g of pyraclostrobin (purity: 98.2%) was applied (semi-occlusive) to 3 rabbits/sex for 4 hours (observation period: 15 days post application). For none of the animals was the mean score (of 24-, 48- and 72-h values) ≥2.3 and ≤4.0 for erythema/eschar or for oedema. See the table below. In most rabbits, erythema (and in some rabbits also oedema) extended beyond the area of exposure. Skin findings were not reversible within 15 days in 2 animals (nos. 3 and 4). In 1 of these rabbits (no. 3) scaling, erythema and oedema extending beyond the area of exposure were noted whereas in the other rabbit (no. 4), only erythema beyond the area of exposure was observed (TOX2000-712: 1998).

***Table**: Skin irritation scores (erythema/oedema) from the OECD TG 404 study (TOX2000-712: 1998) (Table 30 in the CLH report)*

Animal Number	Time after patch removal						Mean (24-72 h)
	1 h	24 h	48 h	72 h	8 d	15 d	
1	2/0	2/1	2/0	0/0	0/0	0/0	1.3/0.3
2	2/0	2/1	2/1	2/0	1/0	0/0	2.0/0.7
3	2/0	2/0	2/0	2/0	3/1	2/1	2.0/0.0
4	2/0	2/0	2/0	1/0	1/0	1/0	1.7/0.0
5	2/0	2/1	2/1	2/1	2/1	0/0	2.0/1.0
6	1/0	2/1	2/0	2/0	2/0	0/0	2.0/0.3

According to the 2nd criterion for classification for skin irritation (category 2), when inflammation persists to the end of the observation period in 2 or more test animals, taking into consideration alopecia (limited area), hyperkeratosis, hyperplasia and scaling, then a test material shall be considered to be an irritant. RAC considers that this criterion is met for pyraclostrobin as inflammation observed in two animals of the OECD TG 404 study persisted to the end of the observation period.

In addition to the skin irritation noted in the GPMT (OECD TG 406), erythema was also observed in all animals in the acute dermal toxicity study (OECD TG 402) and dose-related signs of local irritation were observed at all dose levels in the area of the treated skin in the 28-d repeated dose dermal toxicity study in Wistar rats with pyraclostrobin (OECD TG 410). These studies provide supporting information on the skin irritation potential of pyraclostrobin.

Overall, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Skin Irrit. 2; H315.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Based on an acute eye irritation/corrosion study (OECD TG 405) with pyraclostrobin, the DS proposed no classification for eye irritation.

Comments received during consultation

Two MSCAs commented in support of the DS proposal.

Assessment and comparison with the classification criteria

In the acute eye irritation/corrosion study (OECD TG 405, GLP-compliant and no deviations), 33 mg of pyraclostrobin (purity: 98.2%) was applied to the conjunctival sac of the right eyelid of one male and five female New Zealand White rabbits (observation period: 8 days post application). Only conjunctival effects (redness and swelling) were observed that were fully reversible by 8 days (TOX2000-713: 1998). See table below for mean scores (after grading at 24, 48 and 72 hours).

***Table**: Eye irritation mean scores (of 24, 48 and 72 h values) from the OECD TG 405 study (TOX2000-713: 1998) (Table B.6.2-7 in RAR Vol. 3CA - B.6)*

Animal Number	Opacity	Iris	Conjunctiva		Additional signs
			Redness	Swelling	
1	0.0	0.0	1.3	1.0	Loss of hair at margins of eyelids in all animals.
2	0.0	0.0	2.0	1.0	
3	0.0	0.0	2.0	0.3	
4	0.0	0.0	2.0	0.3	
5	0.0	0.0	1.3	0.7	
6	0.0	0.0	1.3	0.3	

According to the CLP guidance (2017), section 3.3.2.3.2.2 (pg. 310), conjunctival redness and/or swelling mean scores of ≥ 2 in at least 4 out of 6 rabbits would lead to classification for eye irritation in Category 2. In the OECD TG 405 study with pyraclostrobin, only 3 out of 6 rabbits had a mean conjunctival redness score of 2 and the rest of the conjunctival redness scores and all the conjunctival swelling scores were < 2. Thus, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for serious eye damage/eye irritation.**

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS stated in the CLH report that no human or animal data on respiratory sensitisation potential of pyraclostrobin were available for evaluation and thus proposed no classification.

Comments received during consultation

One Company/manufacturer) commented in support of the DS proposal.

Assessment and comparison with the classification criteria

RAC agrees with the DS that **pyraclostrobin warrants no classification for respiratory sensitisation due to lack of data.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Based on no skin sensitisation effects in the GPMT (OECD TG 406) with pyraclostrobin, the DS proposed no classification.

Comments received during consultation

Two MSCAs commented in support of the DS proposal.

Assessment and comparison with the classification criteria

In the skin sensitisation study (GPMT; Magnusson and Kligman method; OECD TG 406, GLP-compliant, no deviations), 20 animals were given intradermal and percutaneous induction doses of 5% and a topical challenge dose of 1% (maximum non-irritant concentration, on days 14 and 21) of pyraclostrobin (purity: 99%, vehicle: 1% Tylose CB 30,000 in aqua bidest). No skin findings were observed after the 1st and 2nd challenges (TOX2000-714: 1998). Thus, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for skin sensitisation.**

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

Summary of the Dossier Submitter's proposal

Several repeated dose toxicity studies covering three species and three routes of exposure are summarised in the CLH report for pyraclostrobin:

- 4 sub-acute studies in rats – 1 each via oral & dermal routes and 2 via inhalation route,
- 3 sub-chronic oral studies – 1 each in rats, mice, and dogs,
- 2 chronic oral studies – 1 each in rats and in dogs,
- 2 carcinogenicity studies – 1 each in rats and in mice,
- 1 one-generation study in rats; and
- 1 two-generation study in rats.

The DS proposed STOT RE 2; H373 (liver and gastrointestinal tract) for pyraclostrobin based on increasing severity of liver effects in rats with prolonged exposure, and consistent observation of duodenal mucosal hyperplasia after sub-chronic repeated exposure in three species and also after sub-acute inhalation exposure in rats.

Blood and spleen were also identified as target organs in the studies, but the DS did not propose classification as the effects in these organs were more pronounced only at doses higher than those resulting in the liver and duodenal effects in rats.

Comments received during consultation

One MSCA and one Company/manufacturer commented but did not state their position on the DS proposal for STOT RE 2 (liver and gastrointestinal tract).

The MSCA questioned why the effects on respiratory tract (atrophy/necrosis of olfactory epithelium at 30 mg/m^3) seen in the 2 sub-acute inhalation studies were not taken into consideration for classification. The DS responded that these effects are instead considered to be local irritation and were used as supporting evidence for the proposed STOT SE 3 classification.

The Company/manufacturer supported the DS view that the duodenal hypertrophy observed at 30 mg/m^3 in one sub-acute inhalation study does not justify STOT RE 1 classification as the severity was low and the effect was not reproducible in the other sub-acute inhalation study.

Assessment and comparison with the classification criteria

In a sub-acute oral study (OECD TG 407, GLP-compliant and no deviations), pyraclostrobin (purity: 94 – 99%) was administered via diet to Wistar rats (5/sex/group) at (M/F) 1.8/2, 9/9.6, 42.3/46.6 and 120/126 mg/kg bw/d (TOX2000-715: 1999). The effects seen are described below.

Liver: ↑ rel. liver weights (M: +15%, F: +26%) and hepatocellular hypertrophy (4 M, 1 F) at high dose. ↓ hepatocellular fat storage (M & F) at mid-high and high dose.

Blood: ↓ red blood cells (RBC) and haemoglobin in females at mid-high and high dose; ↑ prothrombin time in males (mid-high and high dose) and females (high dose); ↑ extramedullary haematopoiesis in spleen at mid-high (4 M, 5 F) and high dose (5 M, 4 F), and ↑ rel. spleen weights in males (+33% at mid-high (not statistically significant) and +66% at high dose) and in females (+32% at mid-high and +48% at high dose).

Duodenum: Mucosal hyperplasia in the duodenum at mid-high (4 M, 2 F) and high dose (4 M, 4 F).

***Table**: Haematological findings in the sub-acute oral study (TOX2000-715: 1999) (adapted from Table B.6.3-3 in RAR Vol. 3CA - B.6)*

Parameter	Sex	Dose mg/kg bw/d (M/F)				
		0	1.8/2	9/9.6	42.3/46.6	120/126
Red blood cells (T/L)	m	8.47	8.12	8.14	8.23	8.22
	f	8.12	7.88	8.11	7.59**	7.35**
Haemoglobin (mmol/L)	m	9.6	9.4	9.6	9.3	8.9
	f	9.5	9.3	9.4	8.8**	8.8**
Prothrombin time (s)	m	28.0	28.2	26.9	28.9**	30.2**
	f	24.9	24.6	24.7	25.7	27.8*

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02

Support for STOT RE classification: The equivalent guidance values for a 28-d oral study are ≤ 30 mg/kg bw/d for Category 1 and ≤ 300 mg/kg bw/d for Category 2. The adverse effects in this study were observed at mid-high (M/F: 42.3/46.6 mg/kg bw/d) and/or high dose (M/F: 120/126 mg/kg bw/d) that correspond to the equivalent guidance value for Category 2.

RAC considers the observed liver effects (↑ rel. weights, ↑ hepatocellular hypertrophy and ↓ hepatocellular fat storage) as adaptive responses.

Effects indicative of haemolytic anaemia were observed in females. However, the changes in blood parameters were < 10% compared to controls. The increase in spleen weight may be correlated to increase in extramedullary haematopoiesis in spleen. However, in the absence of any major changes in haematological parameters, RAC considers that these effects do not merit classification.

The duodenal mucosal hyperplasia is of concern since many animals were already affected at the mid-high dose.

In a sub-acute inhalation study (similar to OECD TG 413, GLP-compliant and no deviations), pyraclostrobin (purity: 98.7%; aerosol – solid dissolved in acetone) was administered (6 h/d, nose-only) to Wistar rats (10/sex/group) at 1, 30 and 300 mg/m^3 (= 0.001, 0.03 and 0.3 mg/L) (ASB2008-5026: 2005).

At 0.3 mg/L, 4 males & 3 females died during days 7 to 24 of exposure period with clinical signs of visually increased respiration, urinous odour and piloerection observed before death. At 0.03 mg/L, the body weight gain of males was reduced by 43%.

Respiratory tract: atrophy/necrosis (minimal to moderate severity) in olfactory epithelium of nasal cavity at 0.03 and 0.3 mg/L.

Liver: No adverse effects reported.

Blood: The DS reported a slight ↑ in white blood cells (WBC) and polymorphonuclear neutrophils (M & F) at 0.3 mg/L. RAC notes that these changes were not statistically significant (Table B.6.3-26 of RAR Vol. 3CA - B.6).

Duodenum: Dose-related ↑ in incidence and severity of duodenal mucosal hyperplasia (M & F) at 0.03 and 0.3 mg/L.

Table: Grading of duodenal mucosal hyperplasia in the sub-acute inhalation study (ASB2008-5026: 2005) (adapted from Table 83 in the CLH report)

	Males [concentration in mg/L]					**Females [concentration in mg/L]**				
Grade	**0 (N)**	**0 (V)**	**0.001**	**0.03**	**0.3***	**0 (N)**	**0 (V)**	**0.001**	**0.03**	**0.3**
0	10	10	10	5	1	9	9	10	5	0
1				5	4		1		5	1
2					3	1				5
3										3
4										1
Average severity grading				[1.0]	[1.3]	[2.0]	[1.0]		[1.0]	[2.4]

N: negative control with conditioned air; V: vehicle control with acetone
The grading of mucosal hyperplasia was assessed by a quantitative mucosal area measurement performed by analysis Doku 3.0. The following gradings were used: grade 0 = duodenal mucosal area up to 6 mm^2; grade 1 = >6-8 mm^2; grade 2 = >8-10 mm^2; grade 3 = >10-12 mm^2; grade 4 = >12 mm^2.
* evaluation of 2 animals in this group was not possible due to advanced autolytic changes

Support for STOT RE classification: The equivalent guidance value for a 28-day inhalation study is ≤ 0.06 mg/L/6h/d for Category 1 and ≤ 0.6 mg/L/6h/d for Category 2.

The duodenal mucosal hyperplasia in this study is of concern. The effects seem more pronounced in females and 1 female showed highest severity grade in the high dose group. However, it should be noted that the evaluation of 2 males in the high dose group was not possible due to autolytic changes.

The effect on olfactory epithelium is also of concern since atrophy/necrosis was observed at mid- and high dose groups.

In another sub-acute inhalation study (similar to OECD TG 413, GLP-compliant and no deviations), pyraclostrobin (purity: 99.02%; aerosol – solid dissolved in acetone) was administered (6 h/d, nose-only) to Wistar rats (10/sex/group) at 3, 10 and 30 mg/m^3 (= 0.003, 0.01 and 0.03 mg/L) (ASB2015-11604: 2014).

At 0.03 mg/L, the body weight gain of males was reduced by 29% (not statistically significant).

Respiratory tract: atrophy/necrosis (minimal to slight severity) in olfactory epithelium of nasal cavity at 0.03 mg/L.

Liver: No adverse effects reported.

Blood: Slight but statistically significant ↓ (up to -5%) in haemoglobin in females at 0.01 and 0.03 mg/L. ↑ abs. weight of spleen in females at 0.03 mg/L but without histopathological changes.

Duodenum: ↑ weight of duodenum in males (abs. up to 35%; rel. up to 33%) and females (abs. up to 40%; rel. up to 34%) at 0.01 and 0.03 mg/L but without histopathological changes (Tables B.6.3 - 39 & 40 & 40 of the RAR Vol. 3CA - B.6).

Support for STOT RE classification: The equivalent guidance value for a 28-day inhalation study is ≤ 0.06 mg/L/6h/d for Category 1.

The effects on blood were minor and the increase in spleen weights were not corroborated by histopathological changes. Therefore, these effects do not merit classification.

The effects on duodenum (↑ weights by >30%) and olfactory epithelium (atrophy/necrosis) are of concern and are consistent with the previous study.

In a sub-acute dermal study (OECD TG 410, GLP-compliant and no deviations), pyraclostrobin (purity: 99%) was administered (6 h/d, semi-occlusive dressing) to Wistar rats (10/sex/group) at 40, 100 and 250 mg/kg bw/d (TOX2000-716: 1999).

No adverse effects on liver, blood or duodenum were reported in this study.

Dose-related signs of local irritation were observed at ≥ 40 mg/kg bw/d. No systemic toxicity was observed at the highest dose.

In a sub-chronic oral study (OECD TG 408, GLP-compliant and no deviations), pyraclostrobin (purity: 98.5%) was administered via diet to Wistar rats (10/sex/group) at (M/F) 3.5/4.2, 10.7/12.6, 35/41, 69/80 and 106/119 mg/kg bw/d (TOX2000-717: 1999).

Liver: ↓ abs. weight in males (-12 to -20%) at ≥ 10.7 mg/kg and ↑ abs. weight in females (+22%) at 119 mg/kg. ↑ incidence and severity of centrilobular hepatocyte hypertrophy. Diminished incidence and/or severity of hepatocellular fat storage (fatty change, diffuse) at ≥ 35/41 mg/kg (M & F).

Blood: ↓ RBC and haemoglobin in females at ≥ 80 mg/kg and ↑ in reticulocytes and in total bilirubin in males (≥ 69 mg/kg) and females (119 mg/kg). ↑ prothrombin time in males (≥ 69 mg/kg).

Spleen: ↑ abs. (+17% at 106 mg/kg) and rel. (+29% at 69 mg/kg and +61% at 106 mg/kg) weight of spleen in males. ↑ abs. (+18 to +58%) and rel. (+22 to +74%) weight of spleen in females at ≥ 41 mg/kg. ↑ severity (M) or incidence (F) of extramedullary haematopoiesis in spleen at ≥ 69/80 mg/kg. ↑ incidence and severity of sinusoid distension and histiocytosis in the spleen (M & F) at ≥ 69/80 mg/kg.

Duodenum: ↑ incidence and/or severity of duodenal mucosal hyperplasia (characterised by increased number of epithelial cells and slightly elongated and broadened villi) in males at ≥ 35 mg/kg and females at 119 mg/kg.

***Table**: Treatment-related haematology findings in the sub-chronic oral study in rats (TOX2000-717: 1999) (adapted from Table 80 in the CLH report)*

Parameter	Sex	Dose in mg/kg bw/d (M/F)					
		0	3.5/4.2	10.7/12.6	35/41	69/80	106/119
White blood cells [G/L]	M	8.41	8.97	8.05	8.92	8.93	9.59
	F	3.90	4.22	4.85	4.69	6.65***	6.55**
Red blood cells [T/L]	M	8.53	8.53	8.79	8.59	8.36	8.22
	F	7.95	7.91	7.95	7.70	7.36***	7.10***
Haemoglobin [mmol/L]	M	9.7	9.5	9.8	9.7	9.5	9.4
	F	9.2	9.3	9.3	9.3	8.7**	8.6***
Reticulocytes [‰]	M	17	17	16	19	24**	33***
	F	14	17	14	13	15	23***
Prothrombin time [s]	M	26.0	26.5	26.4	27.1	28.9***	29.4***
	F	25.6	24.7	25.5	26.1	27.5***	26.3
Total bilirubin [µmol/L]	M	1.69	1.70	1.76	2.20	2.67***	3.29***
	F	2.17	1.93	1.94	1.93	2.69	2.94**
Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02;***p<0.002							

***Table**: Treatment-related histopathological findings in the sub-chronic oral study in rats (TOX2000-717: 1999) (adapted from Table 82 in the CLH report)*

	Males						**Females**					
Dose (mg/kg bw/d)	**0**	**3.5**	**10.7**	**35**	**69**	**106**	**0**	**4.2**	**12.6**	**41**	**80**	**119**
Duodenum # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Mucosal hyperplasia	2	1	1	4	5	10	2	1	2	1	1	10
	[1.0]	[1.0]	[1.0]	[1.3]	[1.2]	[1.9]	[1.0]	[1.0]	[1.5]	[1.0]	[2.0]	[1.4]
Liver # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- hypertrophy, centrilobular	-	-	-	3	6	10	-	-	-	-	-	4
	-	-	-	[1.0]	[1.2]	[1.8]	-	-	-	-	-	[1.0]
- Fatty change, diffuse	10	8	9	6	2	-	4	7	5	2	1	-
	[2.5]	[2.1]	[2.2]	[1.5]	[1.0]	-	[1.5]	[1.4]	[1.4]	[1.0]	[3.0]	-
Spleen # examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Extramedullary haematopoiesis	2	-	3	1	2	3	-	-	3	3	9	9
	[1.0]	-	[1.0]	[1.0]	[1.5]	[1.7]	-	-	[1.7]	[1.7]	[1.3]	[1.8]
- Haemosiderin deposition	10	10	10	10	10	10	10	10	10	10	10	10
	[2.7]	[2.5]	[2.1]	[2.5]	[1.8]	[1.7]	[2.9]	[2.7]	[2.8]	[2.5]	[2.3]	[2.3]
- Sinusoid distension	-	-	-	1	10	8	-	-	-	2	8	10
	-	-	-	[1.0]	[1.4]	[2.0]	-	-	-	[1.0]	[1.1]	[1.7]
- Histiocytosis	-	-	1	3	6	10	-	-	1	2	7	7
			[1.0]	[1.7]	[1.5]	[1.8]	-	-	[1.0]	[1.0]	[1.3]	[1.7]
[ ] average severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The average severity is the sum of the gradings divided by the incidence.												

Support for STOT RE classification: The guidance value for a 90-d oral study is > 10 and ≤ 100 mg/kg bw/d for Category 2.

The changes in liver weights were not consistent between sexes (abs. weight increased in males while it decreased in females). However, histopathological findings in liver up to moderate severity were observed.

Some of the changes in blood were small (the decrease in RBC and haemoglobin, observed only in females, was <10%; the increase in prothrombin time was up to 11%) and some changes were marked but observed only in males (increase in reticulocytes was 41% and total bilirubin was 58%). These changes are indicative of haemolytic anaemia. The changes in spleen weight were corroborated by histopathological findings in spleen. However, since there were no other serious effects along with haemolytic anaemia, RAC considers that the changes in blood do not merit classification.

Histopathological effects in duodenum are of concern as they were observed in males already at a low dose of 35 mg/kg.

In another sub-chronic oral study (OECD TG 408, GLP-compliant and no deviations), pyraclostrobin (purity: 98.5%) was administered via diet to B6C3F1 mice (10/sex/group) at (M/F) 9.2/12.9, 30.4/40.4, 119/162, 274/374 and 476/635 mg/kg bw/d (TOX2000-718: 1998).

Liver: No adverse effects reported.

Blood: ↓ WBC in males at ≥ 274 mg/kg. ↓ haematocrit in males at ≥ 30.4 mg/kg (only -4% at 30.4 mg/kg). ↓ haemoglobin in females at ≥ 374 mg/kg. (Table 85 of the CLH report)

Duodenum:↑ incidence and/or severity (minimal to moderate grade) of duodenal mucosal hyperplasia (characterised by intestinal villi being elongated, partly slightly broadened and branched) at ≥ 119/162 mg/kg. (Table 86 of the CLH report)

Support for STOT RE classification: The guidance value for a 90-d oral study is > 10 and ≤ 100 mg/kg bw/d for Category 2. No adverse effects were observed at 30.4/40.4 mg/kg but at the next dose level of 119/162 mg/kg histopathological findings up to moderate severity were observed in duodenum.

In another sub-chronic oral study (OECD TG 409, GLP-compliant and no deviations), pyraclostrobin (purity: 97.09%) was administered via diet to Beagle dogs (5/sex/group) at (M/F) 2.8/3, 5.8/6.2 and 12.9/13.6 mg/kg bw/d (TOX2000-719: 1999).

Liver: No adverse effects reported.

Blood: The only effect indicative of haemolytic anaemia was increased platelet counts (information on magnitude not available) in females at 13.6 mg/kg.

Duodenum: Slight thickening of the duodenal wall (2 M & 2 F) and duodenal mucosal hypertrophy (2 M & 1 F) at 12.9/13.6 mg/kg.

Support for STOT RE classification: The guidance value for a 90-d oral study is > 10 and ≤ 100 mg/kg bw/d for Category 2. No (statistically significant and/or severe) adverse effects were observed in liver or blood. Gross- and histopathological findings were observed in the duodenum.

In a chronic oral study (OECD TG 452, GLP-compliant and no deviations), pyraclostrobin (purity: 98.7%) was administered for 12 months via diet to Beagle dogs (5/sex/group) at (M/F) 2.7/2.7, 5.4/5.4 and 10.8/11.2 mg/kg bw/d (TOX2000-725: 1999).

At the high dose, vomitus was observed during the first week of administration (3 M, 4 F) whereas diarrhoea occurred in all animals during the entire administration period; at the end of the study, there were no statistically significant changes in food consumption or body weights.

Liver: No adverse effects reported.

Blood: The only effect indicative of haemolytic anaemia was increased platelet counts in males (up to 37%) and in females (up to 30 %, not statistically significant) at 10.8/11.2 mg/kg.

Duodenum: No adverse effects reported.

Support for STOT RE classification: The equivalent guidance value for a 12-month oral study is > 2.5 and ≤ 25 mg/kg bw/d for Category 2. No adverse effects on liver or duodenum and no significant adverse effects on blood were reported in this study.

In a chronic oral study (OECD TG 452, GLP-compliant and no deviations), pyraclostrobin (purity: 97.09%) was administered for 24 months via diet to Wistar rats (20/sex/group) at (M/F) 1.1/1.5, 3.4/4.6 and 9/12.3 mg/kg bw/d (TOX2000-726: 1999).

No adverse effects on liver, blood or duodenum were reported in this study.

There was no test substance-related increase in mortality or clinical signs of toxicity in this study. There were no statistically significant changes in food consumption or body weights.

In the carcinogenicity study in rats (TOX2000-727: 1999), no adverse effects on blood or duodenum were reported. An increased incidence in liver cell necrosis was observed in 10 out of 50 males (vs. 1/50 controls) of the high dose group (9.2 mg/kg bw/d; the equivalent guidance value for a 24-month oral study is > 1.25 and ≤ 12.5 mg/kg bw/d for Category 2).

The DS described the liver findings in the CLH report as follows: the liver cell lesion found in 9 males was characterised by predominantly periportal piecemeal necrosis, often associated with small group or extended bridging necrosis. One animal exhibited moderate focal subcapsular necrosis. Among the 10 males with this lesion, 8 died before study termination, but liver cell necrosis was not regarded as the major cause of death in these animals.

The liver cell necrosis observed in 20% of the high dose males in this study is of concern.

There was no test substance-related increase in mortality or clinical signs of toxicity in this study. Survival rate and other details were lacking. At the high dose, the changes in body weight gain were statistically significant in females (-21.7%) but not in males (-4.9%).

In the carcinogenicity study in mice (TOX2000-728: 1999) and the two-generation reproduction toxicity study in rats (TOX2000-729: 1999), no adverse effects on liver, blood or duodenum were reported.

In the carcinogenicity study in mice, there was no test substance-related increase in mortality and no clinical signs of toxicity were observed. Survival rate and other details are lacking. Reduced body weight (M: -13%; F: -9,5%) and body weight gain (M: -28% M; F: -20%) were observed at 17.2/20.5 mg/kg bw/d.

In the two-generation study in rats, there were no statistically significant changes in food consumption or body weights at the high dose.

In the one-generation reproduction toxicity study in rats (ASB2017-5538: 2002), no adverse effects on liver were reported. Slight indications of anaemia were observed in males and females (< 5% decrease in haemoglobin; Table B.6.6-3 in RAR Vol. 3CA - B.6). In gross pathology investigations, thickening of the duodenal wall was observed in all 10 high dose males (59.1 mg/kg bw/d. The guidance value for an 80-d oral study (approx. exposure period of males in this study) is > 11.25 and ≤ 112.5 mg/kg bw/d for Category 2.

Overall, effects on blood (and/or spleen) indicative of slight haemolytic anaemia were observed in sub-acute oral & inhalation studies in rats; in sub-chronic studies in rats, mice (only above the guidance value for Category 2) and dogs (also in the chronic study); and in the one-generation study in rats. Considering that the effects on blood were not statistically significant and/or severe, RAC agrees with the DS conclusion that these do not warrant classification.

Severe histopathological changes in liver (necrosis) were reported in the carcinogenicity study in rats. Changes in liver weights corroborated by histopathology (hypertrophy and fat storage) were also observed in sub-acute and sub-chronic oral studies in rats. These effects were observed at doses within the (equivalent) guidance values for Category 2. Therefore, RAC agrees with the DS conclusion that the effects on liver with pyraclostrobin warrant classification.

Effects on the duodenum (changes in weight and/or histopathology) were observed in sub-acute oral and inhalation studies in rats, and sub-chronic studies in rats, mice (at just above the guidance value for Category 2 in male mice) and dogs at doses within the (equivalent) guidance values for Category 2. Gross pathological changes (thickening of the duodenal wall) were also observed in sub-chronic study in dogs and in the one-generation study in rats at doses within the (equivalent) guidance values for Category 2. Since the effects were consistently observed (in

short- and long-term studies; in 3 species; via oral and inhalation routes), RAC agrees with the DS and concludes that the effects on duodenum with pyraclostrobin warrant classification.

Moreover, effects on the olfactory epithelium of the nasal cavity (atrophy/necrosis) were observed in both the sub-acute inhalation studies with the more severe effects occurring at (equivalent) guidance values for Category 2. Therefore, RAC concludes that also the effects on nasal cavity with pyraclostrobin warrant classification.

Overall, RAC concludes that **pyraclostrobin warrants classification as STOT RE 2; H373 (liver, gastrointestinal tract, nasal cavity).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Based on the negative results from several standard *in vitro* and one *in vivo* mutagenicity/genotoxicity study with pyraclostrobin, the DS proposed no classification for germ cell mutagenicity.

Comments received during consultation

Two MSCAs and a Company/manufacturer supported the DS proposal. The Company/manufacturer also commented that the *in vitro* micronucleus test in human lymphocytes (ASB2019-10762: 2018) was a range-finding study which explains the guideline (OECD TG 487) deviation of scoring only 1000 cells instead of 2000 (see table further below). The company further noted that the results were negative when it tested pyraclostrobin batches (spiked with low amounts of technical impurities) in 9 *in vitro* micronucleus tests in human lymphocytes (guideline- and GLP-compliant). These studies are available in the confidential volume 4 of the RAR.

Assessment and comparison with the classification criteria

***Table**: Summary table of mutagenicity/genotoxicity tests with pyraclostrobin (Tables 42 and 43 in the CLH report)*

Method, test guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
In vitro				
Bacterial reverse mutation assay OECD TG 471 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 uvrA Concentrations up to 5000 µg/plate; without and with S-9 mix	Negative	TOX2000-720: 1997
Bacterial reverse mutation assay OECD TG 471 GLP-compliant; No deviation	Pyraclostrobin (purity 99.2%)	*S. typhimurium* TA1535, TA100, TA1537, TA98, *E. coli* WP2 uvrA Concentrations up to 5000 µg/plate; without and with S-9 mix	Negative	TOX2003-1219: 2002
HPRT locus mammalian cell mutagenicity test OECD TG 476 GLP-compliant	Pyraclostrobin (purity 98.2%)	Chinese Hamster ovary (CHO) cells	Negative	TOX2000-721: 1998

Method, test guideline, deviations if any	Test substance	Relevant information about the study	Observations	Reference
In vitro				
No deviation		Concentrations up to 20 µg/ml; without and with S-9 mix		
Unscheduled DNA synthesis in primary rat hepatocytes OECD TG 482 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Dose range: 0-1.0 µg/mL	Negative	TOX2000-723: 1998
Chromosome aberration assay in mammalian cells OECD TG 473 GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	Chinese hamster V79 cells Wide range of concentrations up to 25 µg/mL; without and with S-9 mix	Negative	TOX2000-722: 1999
Micronucleus test in human lymphocytes OECD TG 487 (2016) GLP-compliant Deviation: A total of 1000 binucleate cells per concentration or control were scored instead of 2000 cells as recommended in the guideline.	Pyraclostrobin (purity 99.02%)	Human lymphocytes extracted from one donor (31-years-old healthy, non-smoking and non-medicated female) Dose range: 0.228-12.8 µg/mL with 2 exposure periods (4 or 20 h); without and with S-9 mix	Negative	ASB2019-10762: 2018
Cytokinesis-block micronucleus assay in human lymphocytes (G0 phase and proliferating) Peer-reviewed publication No mention of GLP or OECD TG Supplementary data	Pyraclostrobin (purity 99.9%)	Peripheral lymphocytes extracted from 2 healthy donors (aged 25-28; one male/one female; non-smoking and non-medicated) Up to 6.0 µg/mL (G0 phase), up to 0.75 µg/mL (proliferating lymphocytes); no testing with metabolic activation	Positive (proliferating lymphocytes) Equivocal (G0 phase cells)	ASB2015-11605: 2012
In vivo				
Chromosome aberration (bone marrow micronucleus assay) OECD TG 474 NMRI mice, M/F 5/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 98.2%)	0, 75, 150 and 300 mg/kg bw Single oral gavage administration Sampling after 24 or 48 hours Bone marrow exposure demonstrated via radioactivity detection in a separate toxicokinetic study in NMRI mice exposed to a single oral dose of 300 mg/kg bw of radiolabelled pyraclostrobin	Negative	TOX2000-724: 1998

No human data relevant for germ cell mutagenicity is available for pyraclostrobin.

The standard *in vitro* assays (Ames, gene mutation, chromosome aberration and UDS tests) were negative. In a publication (ASB2015-11605: 2012), pyraclostrobin was found to be positive in a micronucleus test in human lymphocytes. However, this publication is considered only supplementary by the DS as it had 'significant deviations' from the corresponding test guideline OECD TG 487. In any case, negative results from the standard *in vitro* (OECD TG 487, GLP-compliant; ASB2019-10762: 2018) and *in vivo* micronucleus tests (OECD TG 474, GLP-compliant; TOX2000-724: 1998) removes concern for chromosome aberrations.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for carcinogenicity as there were no indications in the standard carcinogenicity studies (OECD TG 451, GLP-compliant and no deviations) in Wistar rats and B6C3F1 mice. In a chronic toxicity study (OECD TG 452, GLP-compliant and no deviations) in Wistar rats, Leydig cell tumours (which are common for this strain) were observed but without a dose response and within the range of historical control data (HCD).

Comments received during consultation

Three MSCAs supported the DS proposal. One of these requested more information on the HCD for Leydig cell tumours. The information provided by the DS in response is reflected by RAC further below in its assessment.

Assessment and comparison with the classification criteria

***Table**: Summary table of the carcinogenicity studies and the chronic study with pyraclostrobin (adapted from Tables 46 and 48 in the CLH report by adding general toxicity observations)*

Method, guideline, deviations if any	Test substance, dose levels duration of exposure	Observations	Reference
Carcinogenicity study OECD TG 451 Wistar rats, M/F 50/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 97.09 %) 0, 25, 75, 200 ppm <u>Test substance intake</u> M: 0, 1.2, 3.4, 9.2 mg/kg bw/d F: 0, 1.5, 4.7, 12.6 mg/kg bw/d 24-month oral dietary exposure	No indication of carcinogenic potential. There was no test substance related increase in mortality or clinical signs of toxicity. Survival rate and other details are lacking. Reduced BW (-4% M; -13.7% F) and BWG (-4.9% M; -21.7% F) were observed in the high dose at the end of the study.	TOX2000-727: 1999
Carcinogenicity study OECD TG 451 B6C3F1 mice, M/F 50/sex/group GLP-compliant No deviation	Pyraclostrobin (purity 97.09 %) 0, 10, 30, 120 ppm (M/F), 180 ppm (F only) <u>Test substance intake</u> M: 0, 1.4, 4.1, 17.2 mg/kg bw/d F: 0, 1.6, 4.8, 20.5, 32.8 mg/kg bw/d 18-month oral dietary exposure	No indication of carcinogenic potential. There was no test substance related increase in mortality and no clinical signs of toxicity were observed. Survival rate and other details are lacking. Reduced BW (-13% M; -9,5% F) and BWG (-28% M; -20%F) were observed at 17.2/20.5 mg/kg bw/d.	TOX2000-728: 1999

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance, dose levels duration of exposure</th><th>Observations</th><th>Reference</th></tr>
<tr><td>Chronic toxicity
OECD TG 452
Rat (Wistar)
M/F
20/sex/group
GLP-compliant
No deviations</td><td>Pyraclostrobin (purity 97.09%)
Oral administration via the diet of 0, 25, 75 and 200 ppm (calculated daily substance intake of 0, 1.1, 3.4 and 9.0 mg/kg bw/d for males and 0, 1.5, 4.6 and 12.3 mg/kg bw/d for females) pyraclostrobin for 24 months</td><td>Leydig cell tumour (LCT) was commonly observed in a number of males of all groups including control. No dose-response relationship was observed (see Table below).
<table>
<tr><th>Dose</th><th>0</th><th>25</th><th>75</th><th>200</th></tr>
<tr><td># males</td><td>20</td><td>20</td><td>20</td><td>20</td></tr>
<tr><td># LCT</td><td>9</td><td>12</td><td>11</td><td>8</td></tr>
<tr><td>%</td><td>45</td><td>60</td><td>55</td><td>40</td></tr>
</table>
There were no test substance-related increases in mortality or clinical signs of toxicity.
There were no effects on food consumption and body weight (no stat. sig. changes).</td><td>TOX2000-726: 1999</td></tr>
</table>

No human data relevant to evaluate carcinogenic potential is available for pyraclostrobin.

In the available animal studies, the only indication of carcinogenic potential was observed in the chronic toxicity study in Wistar rats in which there was a dose independent increase in Leydig cell tumours in the low- (60% of males) and mid dose (55%) groups compared to controls (45%). The incidences were lower in the high dose group (40%). The laboratory's HCD showed a range of 30 – 60% for Leydig cell tumours in the 24 chronic toxicity studies performed in Wistar rats between 1990 and 2000. Both the minimum and maximum incidence rates were observed between 1996 and 1997 which is within the 5-year period of the study.

There were no increased incidences of tumours in the standard carcinogenicity studies in Wistar rats and B6C3F1 mice. Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for carcinogenicity.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The following studies were presented in the CLH report for the evaluation of reproductive toxicity of pyraclostrobin

- A two-generation reproduction toxicity study in rats
- A one-generation reproduction toxicity study in rats (dose-range finding study for the above)
- A prenatal developmental toxicity (PNDT) study in rats
- A PNDT study in rabbits
- A maternal toxicity study in rabbits (i.e., a subsequent PNDT study in rabbits but with a focus on investigating maternal toxicity; foetuses were only removed, counted and weighed. There was no evidence of gross malformations).
- The chronic toxicity study in Wistar rats (histopathological effects observed in testes were reported but these were regarded as not treatment-related and secondary to the atrophy and/or the spontaneous Leydig cell tumours common in this strain).

Adverse effects on sexual function and fertility

Based on no relevant adverse effects having been observed in the two-generation reproduction toxicity study (or the dose-range finding one-generation reproduction toxicity study), the DS proposed no classification for adverse effects on sexual function and fertility, and for adverse effects on or via lactation.

Adverse effects on development

The DS proposed Repr. 2; H361d for adverse effects on development observed in the PNDT study in rabbits. Increased post-implantation losses (total and early resorptions) and increased skeletal malformations were observed in the high dose group (20 mg/kg bw/d). While severe maternal toxicity was observed in the treated does, the DS did not consider it to be of sufficient severity to induce foetal findings by a secondary non-specific mechanism.

Comments received during consultation

Three MSCAs and a Company/manufacturer commented. All 3 MSCAs supported the DS proposal but recommended to assess if there was a correlation between the severity of maternal toxicity in individual does and the developmental effects. The DS in response provided information on some of the individual does and concluded that there was no correlation (see information further below in the Assessment section).

The Company/manufacturer commented that should RAC consider classification necessary, then Category 2 is most appropriate (i.e., they supported DS proposal). It provided further input on the developmental findings including additional historical control data and the critical comments concern the severity of maternal toxicity and on the incidence of skeletal malformations (see information further below in the Assessment section).

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

In a two-generation reproduction toxicity study (OECD TG 416, GLP-compliant and no significant deviations affecting the validity of the study[1]), pyraclostrobin (purity: 98.7%) was administered via the diet to Wistar rats (25/sex/group) at 25, 75 and 300 ppm (corresponding to 2.7, 8.2 and 32.6 mg/kg bw/d) (TOX2000-729: 1999).

Parental toxicity was manifested as reduced food consumption (by around 5%) in high dose males and females, slightly decreased body weight/body weight gain in high dose males and females (mostly ≤ 5% and not statistically significant); relative kidney weight in F0 and F1 males statistically significantly increased by 5.6 or 7.0%, respectively. Given the mild parental toxicity, RAC notes that the maximum tolerated dose was not reached in this study.

No treatment-related adverse effects on sexual function and fertility were reported in this study.

As a dose-range finder for the above, a one-generation reproduction toxicity study was performed with pyraclostrobin (purity: 97.09%) that was similar to OECD TG 415 and was GLP-compliant but had significant deviations regarding the number of animals (10/sex/group instead of 20/sex/group) and exposure duration (F0 generation treated for 45 days premating instead of

[1] From day 14 after parturition onwards, food consumption was not determined since the pups themselves began to consume considerable amounts of solid food.

70 days) (ASB2017-5538: 2002). The dose levels were 200, 400 and 600 ppm, corresponding to:

Males: 20.5, 39.9, 59.1 mg/kg bw/d

Females

- premating: 21.3, 42.5, 60.4 mg/kg bw/d

- gestation: 18.3, 35.0, 53.2 mg/kg bw/d

- lactation (post-natal day (PND) 1-14): 29.0, 51.9, 80.2 mg/kg bw/d

Parental toxicity was manifested as:

- statistically significant and dose-related reductions in food consumption (up to 10% in males and 15% in females at some stages of the study) and statistically significant decreases in body weight/bw gain (up to 10 – 20% in males and 6 – 18% in females) at the mid- and high doses;
- anaemia in mid- and high dose males and females (<5% decrease in haemoglobin);
- organ weight changes in parental males: statistically significant decreases of absolute liver (up to 15% reduction) and kidney (by ca 13%) weights as well as the statistically significant increases of relative testes (by up to 21%) and epididymis weights (+22 %) in mid- and high dose groups which were not accompanied by respective changes in relative (liver & kidney) or absolute (testes & epididymis) weights or by any gross lesions in these organs. The only gross pathology finding was a thickening of the wall of the duodenum in all high dose males.

No treatment-related adverse effects that are relevant for classification for sexual function and fertility were reported in this study.

Since no relevant adverse effects were observed in either one- or two-generation studies, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for adverse effects on sexual function or fertility.**

Adverse effects on development

In the two-generation study, the pup body weight was statistically significantly reduced at the high dose (on PND 21: - 10% in F1 males and -12% in F1 females; -15% in F2 males and -13% in F2 females). RAC considers that the pup body weight decreases on PND 21 may partly be due to pups starting to consume the diet from PND 14. However, decreased pup body weight was also observed in F2 animals (but not F1) on PND 7 by ca. 10%.

In F1 animals vaginal opening was delayed by 1.6 days at the high dose which was due to lower body weight gain. "*Both, control and high-dose female F1 animals, had the same body weight (103.0 g ± 10.9 and 103.0 g ± 9.3, respectively) at vaginal opening, however, the high dose rats achieved this weight 1.6 days later due to the lower body weight gain*" (from the attachment submitted with the comment by the company).

Table*: Pup developmental findings in the two-generation study (TOX2000-729: 1999) (Table 58 in the CLH report)*

Parameter	Dose level (ppm)			
	0	**25**	**75**	**300**
F1 - Body weight, day 21, M / F (g)	52.8 / 51.4	53.7 / 51.3	52.8 / 50.3	**47.4**/ 45.2****
F1 - Vaginal opening (days)	31.7	32.1	32.4	**33.3****
F2 - Body weight, day 7, M / F (g)	15.2 / 14.5	14.9 / 14.6	14.7 / 14.2	**13.5**/ 13.1****
F2 - Body weight, day 21, M / F(g)	52.0 / 49.8	52.6 / 50.2	51.4 / 48.9	**45.0**/ 43.5****

Dunnett's test **p< 0.01

In the one-generation study, there was a dose-related statistically significant decrease in pup body weight gain over PND 4 to 21 in all treated groups (up to ca. -40% at the high dose). Although not specifically reported, the pups may have started consuming the diet from PND 14 also in this study and the decrease in body weight on PND 21 may partly be due to this. Nevertheless, pup body weight showed a dose-dependent decrease of up to -33% at the high dose already on PND 14. There was no statistically significant change in body weight gain of the parental females during lactation. Therefore, RAC considers that the decrease in pup body weight (up to -33% on PND 14) supports classification.

***Table**: Pup body weight changes in the one-generation study (ASB2017-5538: 2002) (adapted from Table B.6.6-2 in RAR Vol. 3CA - B.6)*

Dose [ppm]	**0**	**200**	**400**	**600**
Male pup weight [g]				
- day 1 [g]	6.6	6.5	6.6	6.1
- day 4 - pre cull [g]	9.6	9.1	9.2	**8.0****
- day 4 - post cull [g]	9.6	9.1	9.1	**8.2***
- day 7 [g]	15.3	13.9	13.5	**11.6****
- day 14 [g]	30.3	**25.8****	**25.1****	**20.3****
- day 21 [g]	50.2	**41.9****	**39.7****	**31.4****
Male body weight gain				
- day 4 to 21 [g]	40.6	**32.8****	**30.5****	**23.4****
[%]		**-9.2**	**-14.9**	**-42.4**
Female pup weight [g]				
- day 1 [g]	6.4	6.3	6.3	**5.8***
- day 4 - pre cull [g]	9.4	9.0	9.1	**7.8***
- day 4 - post cull [g]	9.4	9.1	9.1	**7.7***
- day 7 [g]	14.7	14.0	13.3	**11.3****
- day 14 [g]	29.8	26.4	**24.6****	**20.1****
- day 21 [g]	47.7	**42.0***	**38.9****	**31.1****
Female body weight gain				
- day 4 to 21 [g]	38.4	**33.0***	**29.8****	**23.3****
[%]		**-14.1**	**-22.4**	**-39.3**
Statistics: *: p≤0.05, **: p≤0.01				

In a prenatal developmental toxicity study in Wistar rats (OECD TG 414, GLP-compliant and no deviations), pyraclostrobin (purity: 98.9%) was administered via gavage to 25 dams/group during gestation day (GD) 6 – 19 at 10, 25 and 50 mg/kg bw/d (TOX2000-730: 1999).

Maternal toxicity was manifested as statistically significantly reduced food consumption (mid dose: -14%; high dose: -27%) on the first days of treatment (GD 6 – 8) resulting in a statistically significantly lower corrected body weight gain in both groups (-22% and -45%, respectively) (see Table 59 in the CLH report).

No treatment related malformations were observed. At the high dose, increases in the incidences of skeletal variations (rudimentary cervical rib with no cartilage; no dose-response relationship,

slightly above the maximum value of the historical control[1] range) and soft tissue variations (dilated renal pelvis; no dose-response relationship, within the range of historical controls) were observed (see Table 64 in the CLH report). RAC considers that the variations observed in this study do not merit classification.

In a prenatal developmental toxicity study in Himalayan rabbits (OECD TG 414, GLP-compliant and no deviations), pyraclostrobin (purity: 98.9%) was administered via gavage to 25 does/group during GD 7 – 28 at 5, 10 and 20 mg/kg bw/d (TOX2000-731: 1999).

Maternal toxicity was severe and manifested as statistically significantly lower food intake (by 64, 79, and 89% at the low-, mid-, and high dose) on the first two days of dosing, resulting in severe initial body weight losses in all dose groups.

Similar findings were observed in the follow-up maternal toxicity study in Himalayan rabbits (TOX2001-471: 2001). Statistically significant decreases in initial food consumption on the first two days of dosing were noted at the mid dose (3 mg/kg bw/da; -26%) and at the high dose (5 mg/kg bw/d; -41%) (see Table 61 in the CLH report).

***Table**: Maternal findings in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) (Table 60 in the CLH report)*

Parameter	Dose level (mg/kg bw/day)			
	0	**5**	**10**	**20**
Food consumption; day 7-8 (g/animal/day)	98.1	**35.7****	**20.4****	**10.5****
Body weight change; day 7-9 (g)	-3.8	**-43.8****	**-85.5****	**-146.3****
Body weight change; days 7-28 (g)	191.5	131.7	**116.0***	**44.1****
Body weight; day 29 (g)	2961	2807	2851	**2748****
Corrected body weight gain over treatment period (g)	-135.7	-142.4	-132.9	-146.8
Gravid uterus (g)	352.6	302.6	**271.2***	**209.6****

Dunnett's test *p<0.05, **p<0.01

Developmental toxicity was manifested as:

- increased post-implantation losses (total and early resorptions) at the mid dose (17.8% vs 6.2% in controls; not statistically significant, below the maximum value of historical control[2] range) and at the high dose (38.6% vs 6.2% in controls; statistically significant, outside historical control range); 2 does in the mid dose and 3 does in the high dose had resorption of the total litter (vs. none in controls; outside the historical control range); live foetuses were statistically significantly reduced at the high dose (4.9 vs 6.9 in controls).
- at the high dose, an increase in total frequency of skeletal malformations (32% vs 25% in controls; not statistically significant but outside historical control[3] range (0 – 17.6%) (NB: also the controls were outside the HCD range)). The skeletal malformations of concern are in particular absent lumbar vertebrae (18.2% vs 4.2% in controls; statistically

[1] Twenty-nine PNDT studies in Wistar rats from the same laboratory and animal supplier performed between 1993 and 1999.

[2] Twenty-three PNDT studies in Himalayan rabbits from the same laboratory performed between 1993 and 2003.

[3] Seven PNDT studies in Himalayan rabbits from the same laboratory performed between 1995 and 1997.

significant in the Cochrane Armitage Trend Test; outside the historical control range (0 - 5.9 %)).

***Table**: Developmental findings in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) (adapted from Tables 66 and 67 in the CLH report)*

Parameter	**Historical control data,** where available **Mean (min – max)**	**Dose level (mg/kg bw/day)**			
		0	**5**	**10**	**20**
Number of pregnant females at terminal sacrifice		24	24	22	25
Implantation sites (mean per litter)		7.4	6.6	6.9	7.0
Post-implantation loss	10.7% (4.6 – 20.1%)	6.2%	10.2%	**17.8%**	**38.6%****
Total resorptions (mean per litter)		0.5	0.6	**1.3**	**2.7****
Early resorptions (mean per litter)	0.4 (0.2 – 0.9)	0.4	0.5	**1.2**	**2.6****
Does with viable foetuses / total number of live foetuses		24 / 166	24 / 145	20 / 123	22 / 107
Resorption of the total litter, number of does affected	0.2# (0 – 1)	-	-	**2**	**3**
Live foetuses (mean per litter)		6.9	6.0	6.2	**4.9****

Dunnett's test *p<0.05, **p<0.01
Occurring in 5 out of 23 studies

***Table**: Skeletal malformations in the prenatal developmental toxicity study in rabbits (TOX2000-731: 1999) (adapted from Table 68 in the CLH report)*

Malformations	**Historical control range**, study mean in brackets	**Dose level (mg/kg bw/day)**			
		0	**5**	**10**	**20**
Total foetal skeletal malformations					
# litters affected/evaluated (%)	0 – 17.6 % (9.3 %)	6/24 (25 %)	4/24 (17 %)	4/20 (20 %)	7/22 (32 %)
Absent lumbar vertebrae					
# litters affected/evaluated (%)	0 - 5.9 % (1.7 %)	1/24 (4.2 %)	1/24 (4.2 %)	1/20 (5 %)	**4/22* (18.2 %)**
Misshapen lumbar vertebrae					
# litters affected/evaluated (%)	0 – 7.1 % (1.7 %)	1/24 (4.2 %)	1/24 (4.2 %)	0/20 (0 %)	2/22 (9.1 %)

*** statistically significant positive trend according to Cochrane Armitage Trend Test p<0.05**

<u>Maternal toxicity vs developmental toxicity</u>

The DS expressed their view on maternal toxicity and post-implantation loss in their response to comment No. 12, as follows (excerpt from the RCOM):

> *"Significant reduction in food consumption and concomitant body weight loss observed early in treatment [i.e., gestation day (GD) 7-9] could have contributed to the severe reproductive outcomes such as increased early resorption and post-implantation loss.*

However, this might not be the only cause for the observed reproductive outcomes as the data of the individual does showed no correlation between reduced food consumption/body weight gain and the severity of reproductive outcomes.

In particular, while all the does at the high dose group (20 mg/kg bw/d) consumed significantly less feed than the control group and exhibited marked body weight loss between GD 7-9, not all of the does experienced significant post-implantation loss. For example, doe nos. 75, 76, 78, 86, and 97 with minimal food consumption (≤ 13.5 g/animal/d between GD 7-8) and dramatic body weight loss (up to -184 g between GD 7-9) had minimal to no post-implantation loss (up to ≤ 12.5%). A similar observation was made also in the mid-dose group (10 mg/kg bw/d). To further demonstrate the lack of correlation, doe no. 73 at the mid-dose group with 100% post-implantation loss exhibited only a slight body weight loss (-55 g) between GD 7-9.

This suggests that maternal toxicity indicated by reduced food consumption and body weight gain might not be the only factor contributing to the post-implantation loss in rabbits. It is our opinion that the degree of maternal toxicity observed is considered not severe enough to trigger such adverse reproductive outcomes like resorption of the total litter."

The DS expressed their view on maternal toxicity and skeletal malformations in their response to comment No. 12, as follows (excerpt from the RCOM):

"Regarding the degree of maternal toxicity observed in the does with lumbar vertebrae malformations found in their litters, there is no clear relationship between maternal toxicity and lumbar vertebrae malformations.

Five does (no. 76, 87, 90, 92 and 93) had at least one foetus with lumbar vertebrae malformations (misshapen or absent). These does did not exhibit any clinical signs of toxicity during gestation and nothing abnormal was detected during necropsy. Three of them (76, 90 and 92) had no post-implantation loss, whereas does 87 and 93 had a post-implantation loss of 25 % and 16.7 %, respectively.

By study termination, does nos. 76 and 90 had more than 10 % decrease in terminal body weight compared to the average terminal body weight of the control group, whereas does nos. 87 and 93 had a slight decrease (5-7 %) in body weight. Doe 92 had a terminal body weight higher than the average terminal body weight of the control.

Altogether, the lumbar vertebrae malformation findings do not appear to be directly correlated with maternal toxicity."

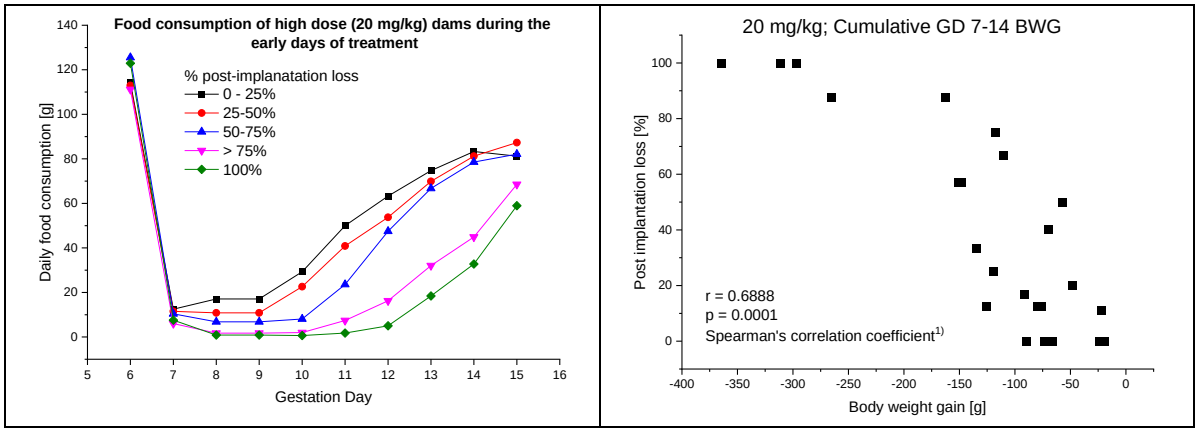
Studies on impact of feed restriction on developmental toxicity are available in New Zealand White rabbits, for e.g., Cappon *et al*., 2005, and Matsuoka *et al*., 2006 (referred to in the attachment submitted with the comment by the company).

In Cappon *et al*. (2005), feed restriction to 15 g/d during GD 7 – 19 led to abortions in 6 of 15 does (vs. none in controls) but no malformations were observed.

In Matsuoka *et al*. (2006), feed restriction to 20 g/d during GD 6 – 18 led to no abortions in the 8 does in the group but there was an increase in post-implantation loss (14.3% vs. 3.1% in controls) and increase in number of does with resorptions (75% vs 12.5% in controls).

In the absence of feed restriction data on Himalayan rabbits, RAC has considered the data on New Zealand White rabbits. The above two studies indicate that severe reduction in food consumption (20 g/d) including the initial days of gestation may lead to abortions or post-implantation loss in rabbits. In the current PNDT study with pyraclostrobin in Himalayan rabbits, there was severe reduction in food consumption in the mid dose (20.4 g/d) and the high dose (10.5 g/d). At high dose, the five does with the most severe body weight gain loss (>150 g) in the group during GD 7 - 14 had the highest post-implantation loss (>80%). See the right panel

of the figure below. Therefore, there is a correlation between the maternal toxicity and the post-implantation loss in these. However, similar to that indicated by the DS in the RCOM (quoted above), there was no correlation in the does with body weight gain loss <150 g. Thus, RAC agrees with the DS that the maternal toxicity might not be the only factor contributing to the post-implantation loss in the rabbit PNDT study with pyraclostrobin, and the increased post-implantation loss supports classification.



1) Spearman C. (1904). "The proof and measurement of association between two things". American Journal of Psychology. 15 (1): 72–101

Figure: Correlation of the extent and duration of food consumption respectively body weight loss during GD 7 to 14 and post-implantation loss in pregnant rabbits treated with 20 mg/kg of pyraclostrobin (copy of figure 4 in the attachment submitted with the comment by the company)

In line with the Cappon *et al*. (2005) conclusion that severe feed restriction did not cause malformations in rabbits, the DS in the RCOM (quoted above) presented that there is no correlation between maternal toxicity (reduced feed consumption and body weight (gain) loss) and malformations in the rabbit PNDT study with pyraclostrobin. The increased incidence of skeletal malformations (absent lumbar vertebrae) at the high dose in this study did not gain statistical significance by group wise comparison but a statistically significant positive trend (Cochrane Armitage Trend Test $p<0.05$) was observed. Therefore, RAC considers that the increased incidence of absent lumbar vertebrae supports classification.

Overall, RAC considers that the following adverse effects provide "some evidence" for developmental toxicity of pyraclostrobin:

- decrease in pup body weight in the one-generation study (up to -33% on PND 14) and the two-generation study (ca. -10% on PND 7 in F2 animals),
- increased post-implantation loss (not completely attributed to maternal toxicity) in the rabbit PNDT study; and
- increased incidence of skeletal malformations (absent lumbar vertebrae) gaining significance by trend test in the rabbit PNDT study.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants classification as Repr. 2; H361d**.

Adverse effects on or via lactation

Classification for effects on or via lactation can be assigned based on:

a) human evidence indicating a hazard to babies during the lactation period; and/or

b) results of one- or two-generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

There is no data on pyraclostrobin fulfilling criteria 'a' or 'c' above. With regard to criterion 'b', adverse effect in the offspring (decreased body weight) during the lactation period was observed in the one-generation study. However, there is no clear evidence of whether this effect is due to pre-natal exposure or due to transfer in the milk or an adverse effect on the quality of the milk.

Therefore, RAC agrees with the DS and concludes that **pyraclostrobin warrants no classification for adverse effects on or via lactation.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Pyraclostrobin is an active substance in plant protection products. In addition to its fungicidal effects, pyraclostrobin shows also physiological effects leading to further increased yield and quality as well as improved tolerance against biotic and abiotic stress in many crops. The substance is currently included in Annex VI of Regulation (EC) No 1272/2008 with classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) with M-factor of 100 for both hazard classes.

The DS proposed to classify the substance as:

- **Aquatic Acute 1 (H400) with M-factor of 100** based on a 96h EC_{50} value of 0.00416 mg/L for the invertebrate *Americamysis bahia*.
- **Aquatic Chronic 1 (H410) with M-factor of 100** based on 31d NOEC value of 0.000365 mg/L for the invertebrate *Americamysis bahia.* The substance has a high bioaccumulation potential and is not rapidly degradable.

Degradation

A hydrolysis study according to OECD TG 111 and EPA 161-1 and in compliance with GLP was run at pH 4, 5, 7 and 9 and at 50 °C and at pH 5, 7 and 9 and 25 °C in the dark in sterile aqueous buffered solutions. Hydrolysis was pH dependent. The substance is stable at pH 5 and 7. Under alkaline conditions (pH 9) a very slow degradation of the substance was observed at 25 °C. Only at high temperatures (50 °C) and under alkaline conditions (pH 9) a comparatively faster degradation was observed, but this does not represent common environmental conditions.

The study on direct aqueous photolysis of pyraclostrobin in sterile aqueous buffered solutions at pH 5 at 22±1 °C was conducted according to BBA IV, 6-1, OECD Draft Test Guideline "Photo-transformation of Chemicals in Water" Part A. During direct photolysis, a very fast degradation of the substance was observed. A large number of degradation and rearrangement products occurred, only some of them being stable under simulated environmental conditions. The molar mass and/or structure of 33 metabolites could be determined. Five of the metabolites (BF 500-11, BF 500-13, BF 500-14, BF 500-15 and 500M58) occurred once or several times with amounts >10 % TAR. Mineralisation after 25 days for two labels were 22% TAR and 4% TAR. In

the dark control, no degradation was observed. The photolytic half-life for pyraclostrobin was calculated to be 0.06 days under study conditions. The quantum yield of pyraclostrobin was estimated to be 0.217.

One ready biodegradability test was available according to EEC 92/69, OECD TG 301F and ISO 9408 and compliance with GLP. The degree of biodegradation (% biological oxygen demand/theoretical oxygen demand) over the 28-day test duration was in the range 0-10%. The substance is therefore not readily biodegradable under test conditions.

In an aerobic mineralisation in surface water study performed according to OECD TG 309 the degradation of pyraclostrobin was studied in a pelagic and suspended solid test under aerobic conditions for 63 days at 20 °C in the dark. In the pelagic test, pyraclostrobin was degraded very slowly. More than 78–97% TAR was still detectable as unchanged parent after 63 days. The degradation of pyraclostrobin was characterized by slow hydrolysis and formation of low amounts of cleavage products, of which BF 500-5 occurred at 5.6-10.9% TAR. All other peaks never exceeded 2.1% TAR. In the suspended solid test, pyraclostrobin dissipated quickly from the water phase (< 45% TAR after 3 days) and adsorbed to the solid particles floating in the water. In the water phase of the suspended solid test, the hydrolysis product BF 500-5 (7.7% TAR), unidentified substance (5.8% TAR) and others (≤ 3.8% TAR) were detected. In the sediment extracts of the suspended solid test, the following metabolites were formed: BF 500-3, BF 500-6 (≤ 2.3% TAR), BF 500-7 (≤ 2.6% TAR) and other components (≤ 0.5% TAR). The formation of volatiles in both test variants was generally low (< 5% TAR), irrespective of test concentration or label position. Overall, the degradation of pyraclostrobin was characterized by a low mineralization rate in both test variants irrespective of test concentration or label position. The amount of $^{14}CO_2$ never exceeded 5% TAR within 63 days. The $DegT_{50}$ values in the pelagic test ranged from 410 to 458 days for pyraclostrobin and from 11 to 29 days for BF 500-3. In the suspended solid test, the $DegT_{50}$ for pyraclostrobin for the whole system ranged from 26 to 28 days, while the $DisT_{50}$ for the water compartment range from 7 to 10 days and for the suspended sediment from 44 to 47 days. The $DisT_{50}$ for BF 500-5 was 103 days when calculated with the high test concentration. For the low test concentration, no adequate fit was achieved. In general, pyraclostrobin hydrolyses only slowly under the pelagic test conditions, but adsorbs quickly to suspended solids, when available, and is then further degraded by formation of bound residues.

The distribution and degradation of radiolabeled pyraclostrobin was studied in two sediment-water systems, taken from a pond (System A) and a pond-like side arm of a river (System B) up to 100 days in dark. The study was conducted according to the SETAC Europe, BBA IV, 5-1, US-EPA, Subdivision N, 162-4 (dark study). The radioactivity moved quite fast from the water to the sediment. The radioactivity in the water decreased to less than 25% TAR within 7 days in system A and within 2 days in system B. A further decrease to less than 3% TAR after 100 days was observed in both systems. In the sediment a corresponding increase was seen which accounted for more than 90% TAR at the end of the incubation period. Low mineralization was observed in both test systems and accounted at a maximum of 4.6% of applied radioactivity and no other volatile degradants were detected. High amounts of bound residues were formed in the sediment which accounted for up to 61.8% TAR in system A and 54.1% TAR in system B. No detectable amounts of pyraclostrobin were released from the bound residues. The following three metabolites were identified in the study: BF 500-3 accounted for a maximum of 67.6% AR at day 14 (1.9% in water, 65.7% in sediment, System B), BF 500-6 accounted for a maximum of 6.5% AR at days 61 and 100 (only in sediment, System A) and BF 500-7 accounted for a maximum of 6.3% AR at day 61 (only in sediment, System A, only once > 5%). The kinetic evaluation of the study following SFO kinetics indicated that pyraclostrobin degraded with DT_{50} values for the whole system of 23.3 days and 26.8 days and DT_{90} values for the whole system of 77.4 days and 89.1 days for the two labels in system A. The geometric mean DT_{50} value is 25.0 days. No reliable

endpoints could be derived for System B. The dissipation from the water phase followed biphasic kinetics in both systems, with $DissT_{50}$ < 3 days and $DissT_{90}$ < 30 days. The dissipation from the sediment followed SFO in system A, with $DissT_{50}$ < 30 days and $DissT_{90}$ < 100 days.

The DS concluded that pyraclostrobin is considered to be not rapidly degradable in the aquatic environment, for classification and labelling purposes.

Bioaccumulation

For pyraclostrobin, the measured octanol-water partition coefficient (log P_{OW}) determined according to OECD TG 117 (HPLC method) was 3.99. The effect of pH was not investigated since there was no dissociation in water.

In a study performed according to OECD TG 305 and EPA 165-4 the bluegill sunfish (*Lepomis macrochirus*) were tested in a flow-through system at nominal concentration of 300 µg/L of ^{14}C-pyraclostrobin for 37 days followed by a 14-day depuration period for the chlorphenyl label and a 21-day phase for the tolyl label. The bioconcentration factor (BCF) for whole fish normalized for a lipid content of 5% was 712 for the chlorphenyl label and 776 for the tolyl label.

The DS concluded that pyraclostrobin has a potential for bioaccumulation as BCF in fish is above the cut-off value of 500 given in the CLP Regulation.

Aquatic Toxicity

Reliable aquatic toxicity tests for both acute and chronic aquatic toxicity are available for all three trophic levels.

<u>Acute toxicity</u>

The summary of the relevant information on acute aquatic toxicity is provided in Table 143 of the CLH report.

For fish, seven acute toxicity studies with five different fish species were available. Rainbow trout *Oncorhynchus mykiss* was the most sensitive fish species tested in the acute studies performed according to EPA 850.1075, 72-1, with mean measured 96h LC_{50} value of 0.00616 mg/L.

Five acute toxicity studies with different taxonomic groups were provided for aquatic invertebrates. The lowest study value, according to EPA 850.1035, EPA 72-3(b), resulted in a 96h LC_{50} of 0.00416 mg/L (mean measured) for saltwater mysid *Americamysis bahia*.

Six acute toxicity studies with five different algae species were available. The freshwater diatom *Navicula pelliculosa* was the most sensitive species tested in algae acute studies performed according to EPA 123-2, EPA 850.5400, with initial measured 72h E_rC_{50} of 0.011 mg/L.

There was one toxicity study available for aquatic plants, conducted according to EPA 123-2, EPA 850.4400 and OECD 221, with mean measured 14d E_rC_{50} value of >1.077 mg/L for *Lemna gibba*.

From the available acute aquatic toxicity data for pyraclostrobin, the DS concluded that toxicity to aquatic organisms for all three trophic levels is below 1 mg/L. Invertebrates are the most acutely sensitive taxonomic group, therefore the acute aquatic classification proposed by the DS was based on saltwater mysid *Americamysis bahia* with 96h LC_{50} of 0.00416 mg/L; experimental information for acute fish also fall within the same concentration range, supporting, thus, the classification conclusion. The DS proposed **Aquatic Acute 1** (H400) with **M-factor of 100** ($0.001 < L(E)C_{50} \leq 0.01$ mg/L).

Chronic toxicity

The summary of the relevant information on chronic toxicity is provided in Table 184 of CLP report.

There are four long-term toxicity studies with three different fish species available. Rainbow trout *Oncorhynchus mykiss* was the most sensitive fish species tested in the chronic studies performed according to OECD 210, with mean measured 98d NOEC value of 0.00235 mg/L.

Five chronic toxicity studies with different taxonomic groups were provided for aquatic invertebrates. The saltwater mysid *Americamysis bahia* was the most sensitive species tested in invertebrate chronic studies performed according to EPA 850.1000, EPA 850.1350, EPA 72-4, with a mean measured 31d NOEC of 0.000365 mg/L.

Six chronic toxicity studies with five different algae species were available. The freshwater diatom *Navicula pelliculosa* was the most sensitive species tested in algae chronic studies performed according to EPA 123-2, EPA 850.5400, with initial measured 120h NOE_rC of 0.00118 mg/L.

There was one study available for aquatic plants, conducted according to EPA 123-2, EPA 850.4400 and OECD 221, with a mean measured 14d E_rC_{10} value of 0.82 mg/L for *Lemna gibba*.

Based on the results from the long-term aquatic toxicity studies with pyraclostrobin, the DS concluded that chronic toxicity to aquatic organisms for all three trophic levels is below 0.1 mg/L. Invertebrates are the most sensitive taxonomic group. Therefore, the chronic aquatic classification proposed by DS was based on the saltwater mysid *Americamysis bahia* toxicity study with 31d NOEC of 0.000365 mg/L. The DS proposed **Aquatic Chronic 1**, with **M-factor of 100** (0.0001 < NOEC ≤ 0.001 mg/L) along with the understanding that the substance is not rapidly degradable and has a high bioaccumulation potential.

Comments received during consultation

An MSCA, a company-manufacturer and a National Authority provided comments. The MS agreed with the proposed classification for environmental hazards by DS

Regarding aquatic toxicity, the company-manufacturer suggested some changes in endpoints and studies reliability. Comments were provided on the following acute fish studies (*Lepomis macrochirus* (14F0494/965179: 1998), *Cyprinus carpio* (11F0494/965178: 1998) and *Pimephales promelas* (18F0494/96E001: 2014)) which were considered of lower reliability by the DS, due to the fish size which was larger then recommended in the OECD TG 203.

Also, it was pointed out that bluegill study *Lepomis macrochirus* (1947-BA: 2000) conducted with smaller individuals produced essentially the same LC_{50} as the study with larger juvenile fish *Lepomis macrochirus* (14F0494/965179: 1998) that was criticized. DS pointed out that none of the studies was considered invalid or implausible because of the length deviation, and they were all considered relevant for classification purposes. However, due to the deviation from the guideline recommendation, and the associated uncertainty in sensitivity, DS retain the assessment to consider the studies reliable with restrictions.

In the view of the Company-manufacturer in case of acute toxicity study with *Americamysis bahia*, which was selected as key study for classification, the 48h LC_{50} value instead of 96h LC_{50} value should be used for classification as this is in line with Regulation (EC) 1272/2008 (section 4.1.2.7.1, Table 4.10) for crustaceans. DS responded that according to the OCSPP 850.1035 guideline, the 96h LC_{50} is the primary endpoint to be derived from acute toxicity tests on mysids.

As the 48h LC_{50} and 96h LC_{50} are within the same order of magnitude, the choice of relevant endpoint does not influence the classification of the substance.

Also, it was pointed out that new and fully valid and reliable acute toxicity study with *Navicula pelliculosa* (Eckenstein, 2018) contradicts the low endpoint of the less reliable acute toxicity study with *Navicula pelliculosa* (Boeri, 2000) which was selected as key study for algae. Therefore, company-manufacturer suggested using the acute toxicity study with *Ankistrodesmus bibraianus* (Backfisch and Englert, 2018) as a key study for algae. DS pointed out that the reliability of the *N. pelliculosa* study (Boeri, 2000) was also discussed in the renewal assessment report. The RMS judged the study as valid and relevant, although some shortcomings were identified.

The company-manufacturer is of the opinion that the NOEC for F_0 male body weight in the chronic toxicity *Americamysis bahia* study (Dinehart, 2013) is of questionable value. It was proposed, instead, the NOEC of 0.00128 mg/L for F_0 reproduction as relevant endpoint in the chronic mysid study, leading to a chronic M-factor of 10. The DS highlighted that the chronic mysid study was discussed during the preparation of the renewal assessment report for pyraclostrobin and the effects on the weight of males at day 31 were considered biologically relevant, therefore the DS considered the NOEC of 0.000365 mg/L as relevant endpoint, leading to a chronic M-factor of 100.

Also, the National Authority commented on the 31-day study on *Americamysis bahia* (Dinehart, 2013) questioning the reliability of the NOEC endpoint for male weight. The DS confirmed that NOEC for *A. bahia* was used as relevant long-term invertebrate endpoint for the risk assessment.

It was asked whether it is possible to recalculate a 72-hour mean measured NOE_rC or E_rC_{10} for the study on *Navicula pelliculosa* (Boeri, 2000) considering the issues identified regarding the analytical verification of test concentrations. The DS recalculated the effect concentrations for 72h and pointed out that E_rC_{10} of 0.000257 mg/L is extrapolated outside the tested concentration range and that the confidence intervals of the E_rC_{10} and E_rC_{20} partially overlap, most likely due to the rather flat concentration-effect relationship observed in the study; It was also mentioned that this indicates that the calculated E_rC_{10} is of low reliability and that the interpretation of the study on *N. pelliculosa* would only affect the proposed classification and M-factor if the RAC does not agree with NOEC for A*. bahia*.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider pyraclostrobin as not rapidly degradable:

- The substance was stable to hydrolysis.
- The substance is not readily biodegradable. Biodegradation in the OECD TG 301 F test was 0-10% after 28 days which is below the pass level of 60 % of the test.
- In the surface water simulation test the mineralization was low (< 5% TAR) and DT_{50} were 410 to 458 days in pelagic test and 26 to 28 days for whole system in suspended solid test.
- The DT_{50} in the whole system in a water/sediment system study (System A/pond) for two labels were 23.3 days and 26.8 days (geometric mean 25.0 days). Low mineralization was observed in both test systems (max. 4.6% TAR). Three main metabolites were formed, namely BF 500-3, BF 500-6 and BF 500-7.

Bioaccumulation

RAC agrees with the DS that pyraclostrobin has a potential for bioaccumulation based on the available bioconcentration study in bluegill sunfish showing a BCF (whole fish, lipid normalized) value of 712 (chlorphenyl label) and 776 (tolyl label), which are above the CLP Regulation threshold of 500. In addition, the measured log P_{OW} of 3.99 is very close to CLP criterion of 4.

Aquatic toxicity

RAC is of the opinion that in the case of the acute toxicity study with *Americamysis bahia* (Boeri, 2000) the 96h endpoint (LC_{50}) should be used for classification because 96h exposure is in accordance with OCSPP 850.1035 guideline. The 96h exposure duration is also in line with Guidance on the Application of the CLP Criteria, section I.2.2.1 (Version 5.0, July 2017) where it is indicated that "*For other crustacea, such as mysids or others, duration of 96 hours is typical*".

In relation to the acute toxicity studies with algae, RAC is of the view that the endpoint 72h ErC50 of 0.011 mg/L from the acute toxicity study with *N. pelliculosa* (Boeri, 2000) should be used as the lowest endpoint for algae species, as the study was considered valid and relevant in both RAR and the CLH study summery although there were some shortcomings. However, RAC noted that the algae are not the most sensitive trophic level.

RAC considers the endpoint male weight from the 31-day chronic toxicity *Americamysis bahia* study (Dinehart, 2013) (NOEC of 0.000365 mg/L) a relevant endpoint for chronic hazard classification. RAC recognizes that the male weight was significantly affected by the pyraclostrobin, and the concentration-response relationship was observed at the three highest test concentrations. In addition, the NOEC based on male weight was agreed for use in risk assessment (RAR).

Acute toxicity

RAC is of the opinion that adequate acute toxicity data are available for all three trophic levels. Invertebrates are the most sensitive group and the lowest acute 96 h EC50 value of 0.00416 mg/L for *Americamysis bahia* is below the classification threshold value of 1 mg/L. RAC concludes that a **classification as Aquatic Acute 1 (H400) with an M-factor of 100** ($0.001 < EC_{50} \leq 0.01$ mg/L) is warranted.

Chronic toxicity

RAC is of the opinion that adequate chronic toxicity data are available for all three trophic levels. Invertebrates are the most sensitive group and the lowest chronic 31d NOEC value of 0.000365 mg/L for *Americamysis bahia* is below the classification threshold value of 0.1 mg/L. RAC concludes that a **classification as Aquatic Chronic 1 (H400) with an M-factor of 100** ($0.0001 < NOEC \leq 0.001$ mg/L for a non-rapidly degrading substance) is warranted.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The hazard to the ozone layer was not addressed by the DS but it was open for consultation.

Comments received during consultation

One company-manufacturer pointed out that based on the very low pyraclostrobin vapour pressure (2.6 x 10-8 Pa at 20°C) and the very short half-life in air (less than 0.1 days according to Atkinson, AOPWIN program version 1.88), a risk for ozone layer depletion can be excluded.

Assessment and comparison with the classification criteria

Pyraclostrobin is not expected to remain stable in the air based on the very short half-life of less than 0.1 days, data provided in the consultation by the company-manufacturer. Due to its low half-life in the atmosphere combined with a low vapour pressure (2.6 x 10^{-8} Pa at 20°C) indicating low volatility and resulting in a low value for the Henry's Law constant (5.31 x 10^{-6} Pa m^3/mol at 25°C), pyraclostrobin is considered not to be subject to transport via air or cause hazard to ozone layer. RAC obtained the data for Henry's Law constant from the following web page http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/564.htm, available 10.6.2022.

Therefore, RAC is of the opinion that **no classification is warranted for hazards to the ozone layer**.

Additional references

Cappon, G.D., Fleeman, T.L., Chapin, R.E. and Hurtt, M.E. 2005. Effects on feed restriction during organogenesis on embryo-fetal development in rabbit. Birth Defects Research (Part B) 74:424-430.

Matsuoka, T., Mizoguchi, Y., Serizawa, K., Ishikura, T., Mizuguchi, H. and Asano, Y. 2006. Effects of stage and degree of restricted feeding on pregnancy outcome in rabbits. The Journal of Toxicological Sciences, Vol. 31, No. 2, 169-175.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).