Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole

EC Number: 266-275-6
CAS Number: 66246-88-6

CLH-O-0000007383-73-01/F

Adopted

30 November 2023

30 November 2023

CLH-O-0000007383-73-01/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 on **30 November 2023** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole**

EC Number: **266-275-6**

CAS Number: **66246-88-6**

Rapporteur, appointed by RAC: **Brendan Murray**

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

Administrative information on the opinion

Norway has submitted on **3 August 2022** 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 **22 August 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 October 2022**.

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 following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

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	613-317-00-X	penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole	266-275-6	66246-88-6	Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H361d H302 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H302 H410		M=1 M=1	
Dossier submitters proposal	613-317-00-X	penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole	266-275-6	66246-88-6	**Retain** Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2	**Retain** H361d H302 H400 H410 **Add** H373 (liver)	**Retain** GHS08 GHS07 GHS09 Wng	**Retain** H361d H302 H410 **Add** H373 (liver)		**Retain** M=1 M=1 **Add** oral: ATE = …	
RAC opinion	613-317-00-X	penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole	266-275-6	66246-88-6	**Retain** Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 **Add** STOT RE 2	**Retain** H361d H302 H400 H410 **Add** H373 (liver)	**Retain** GHS08 GHS07 GHS09 Wng	**Retain** H361d H302 H410 **Add** H373 (liver)		**Retain** M=1 **Modify** M=10 **Add** oral: ATE = 970 mg/kg bw	
Resulting Annex VI entry if agreed by COM	613-317-00-X	penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole	266-275-6	66246-88-6	Repr. 2 Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361d H302 H373 (liver) H400 H410	GHS08 GHS07 GHS09 Wng	H361d H302 H373 (liver) H410		M=1 M=10 Oral ATE: 970 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The active substance Penconazole is a fungicide used in agriculture for control of powdery mildews in various crops such as grape, pome fruits, stone fruits, strawberry, cucumber, and other vegetables, which has an existing entry in Annex VI of CLP (the current harmonised classification is: Acute Tox. 4; H302 and Repr. 2; H361d).

The DS proposal in the CLH report for human health hazards seeks to update the existing harmonised entry with an additional proposal for classification for specific target organ toxicity – repeated exposure (STOT RE) (i.e., STOT RE 2; H373 (Liver)). All hazard classes have been addressed in the CLH report.

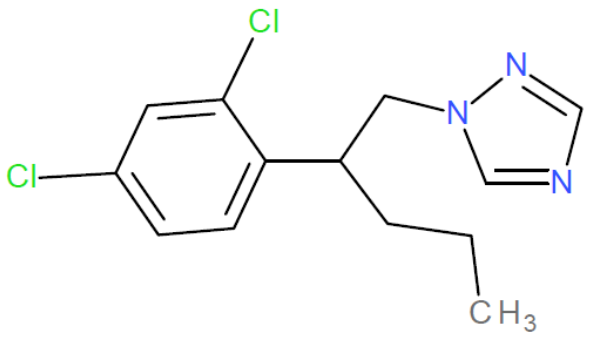

***Figure 1**: Penconazole: Basic structure*

Penconazole belongs to the triazole class of substances and its mode of action is similar to other triazoles (i.e., sterol demethylation inhibitors (DMIs)). Penconazole acts on the fungal pathogen during penetration and haustoria formation. Its main biochemical mode of action is the inhibition of cytochrome P-450 sterol 14α-demethylase (P-45014DM), a key enzyme of the sterol biosynthetic pathway of fungi. Interference with sterol biosynthesis leads to disruption of membrane function, leakage of cytoplasmic contents and hyphal death.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification of penconazole for physical hazards. This was based on negative outcomes where testing occurred, and where the absence of data for certain endpoints was noted.

- Penconazole was not considered to be an explosive substance. The explosive properties of penconazole (TC of purity 98.1% w/w) were studied using Test 2(b); Koenen test, and Test 2(c)(i; Time/Pressure test) as outlined in ST/SG/AC.10/11/Rev.7. In agreement with CR (EU) No 1272/2008 (p. 52), Test 2(a), i.e., UN gap test, was waived based on Penconazole's decomposition energy of 584 J/g (< 800 J/g) determined by using method ASTM E537 (Differential Scanning Calometry). Penconazole's exothermic decomposition energy and the negative outcome of the Koenen test and the Time/Pressure test, concludes the substance is too insensitive for acceptance into Class 1 (ST/SG/AC.10/11/Rev.7).

- Penconazole was not considered to be a flammable substance. The flammable properties of penconazole (TC of purity 98.1% w/w) were studied using Test N.1 (test method for flammable solids), as referred to in ST/SG/AC.10/11/Rev.7. The full test programme was not required as the full burning time over 200 mm was less than 2 minutes.
- No information on self-reactivity for penconazole was provided. Because the heat of decomposition is above 300 J/g, a SADT test was commissioned, however it was not completed and submitted in time for consideration for the CLH report/harmonised classification and labelling procedure.
- No data on self-ignition for penconazole was provided. However, penconazole has been handled in air within studies available in the CLH dossier and there were no reports of self-ignition. Therefore, penconazole shall not be classified as pyrophoric solids.
- No data on self-heating for penconazole was provided. However, penconazole showed a melting range starting at 60°C and was completely molten at 61°C.
- No data on emission of flammable gases was provided. The chemical structure of penconazole does not contain metals or metalloids and no incidences of violent reaction and emission of flammable gases have been reported when penconazole has been handled in water in other studies.
- The oxidative properties of penconazole (TC of purity 98.1% w/w) were studied using Test O.1 (test for oxidising solids), as outlined in ST/SG/AC.10/11/Rev.7. Penconazole, in the 4:1 and 1:1 sample-to-cellulose ratio (by mass), exhibited a mean burning time less than that of a 3:7 mixture (by mass) of potassium bromate and cellulose, i.e. Category 3 in Table 2.14.1 in CR (EU) No 1272/2008).
- Penconazole is not an organic peroxide.
- No information on corrosivity to metals for penconazole was provided. The DS accepted a waiver for corrosivity on the basis that penconazole is a solid.

Penconazole (technical grade) is an off-white lumpy or non-homogeneous powder. The melting point was measured between 60.3 °C to 61.0 °C and the boiling point was measured at > 360 °C at 101.325 kPa. The vapour pressure was measured at 0.224 mPa at 25°C and 0.094 mPa at 20°C. Penconazole has a calculated pKa of 1.51 at 20°C. Solubility in water is 77 mg/L at 20 °C. In organic solvents the substance is soluble in acetone, dichloromethane, ethyl acetate, methanol, toluene (all > 500 g/L), octanol (350 g/L) and hexane (1 g/L). The partition coefficient n-octanol/water was measured to be 3.8 at 20°C.

Comments received during consultation

One MSCA submitted a comment during the public consultation. The MSCA rejected the waiving of corrosivity studies on the corrosive properties of penconazole, which had been granted on the basis that penconazole is a solid. The MSCA requested re-consideration of the waiver based on criteria for corrosivity to metals described in the section 2.16 of Annex I to the CLP Regulation, while acknowledging that no adequate corrosivity to metal test is available to solid substances.

The DS maintained that the waiver is in line with the Guidance on Application of the CLP Criteria[1] (version 5.0; 04.07.2017; Section 2.16).

Assessment and comparison with the classification criteria

The DS did not propose classification for physical hazards. RAC summarised the available information and conclusions regarding all physical hazards and agreed with the DS. Therefore, RAC concluded that **no classification is warranted** for physical hazards.

***Table**: Summary of physical hazard data and classification conclusions*

Hazard Class	Chapter in CLP criteria Guidance	Comments	Conclusion
Explosives	2.1	Explosivity investigated using Test 2 (b) (Koenen Test) and Test 2 (c) (Time/Pressure Test) as outlined in ST/SG/AC.10/11/Rev.7. Test 2(a) (UN gap test) was waived.	No classification, conclusive.
Flammable solids	2.7	Negative screening Test N.1.	No classification.
Self-reactive substance/mixture	2.8	The heat of decomposition is above 300 J/g, a SADT test was commissioned but is not available.	No classification due to lack of data.
Pyrophoric solids	2.10	No test data. Experience in handling is sufficient for waiving a test (CLP, Annex I, 2.10.4.1).	No classification, conclusive.
Self-heating substance/mixture	2.11	No data on self-ignition. Melting point well below 160°C is sufficient for waiving a test (ECHA-17-G-21-EN).	No classification.
Water-reactive - emits flammable gases	2.12	No test data. No reports of violent reaction and emission of gas on contact with water from handling experience. The molecule does not contain metals or metalloids (CLP, Annex I, 2.12.4.1).	No classification, conclusive.

[1] *"There is no reference in the definition (CLP Annex I, 2.16.1) to the physical state of the substances or mixtures that need consideration for potential classification in this hazard class. (…). According to the classification criteria only substances and mixtures for which the application of the UN Test C.1 (described in part III, Section 37.4.1.1 of the UN-MTC) is relevant and needs to be considered. Application of classification criteria in the UN-MTC, Section 37.4 excludes solids, while 'liquids and solids that may become liquids (during transport)', have to be considered for such a classification.*

The wording 'solids that may become liquids' was developed for UN RTDG Model Regulations classification purposes and needs further explanation. Solids may become liquids by melting (due to increase in temperature). Solids having a melting point lower than 55 °C (which is the test temperature required in UN Test C.1) must then be taken into consideration (…).

Non-testing data

Following parameters are helpful to evaluate corrosive properties before testing:

- *melting points for solids (…)"*

Oxidising solids	2.14	Negative UN Test. O.1. The criteria for Categories 1 and 2 are not met and burning time of 2:3 and 3:2 mixture (by mass) of potassium bromate and cellulose is waived.	No classification, conclusive.
Corrosive to metals	2.16	Not applicable.	No classification.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral route

The DS proposed the following classification for penconazole (ISO) 1-[2-(2,4-dichlorophenyl)pentyl]-1H-1,2,4-triazole with respect to acute oral toxicity, Cat 4, H302. This is in line with the harmonised classification registered in ATP 006. RAC does not propose a change to this classification.

There were four acute oral studies with penconazole available, the guideline was not reported for any because the studies predate the OCED guideline 401 of 1981 (One rat, one hamster, one rabbit and one mouse, table 7 of the CLH report).

Study 1 (*Anon., 1980,* Rat)

The acute oral LD_{50} was found to be oral LD_{50} (combined sexes) = 2125 mg/kg bw, for males only: 1000 mg/kg bw < LD_{50} < 2000 mg/kg bw. In this rat study the doses administered were toxic leading to deaths in 5/5 male and 4/5 female rats at 4000 mg/kg bw, and 3/5 males and 0/5 females at 2000 mg/kg bw.

Study 2 (*Anon.*, (1980a), Hamster)

The acute oral LD_{50} for combined sexes ~ 5000 mg/kg bw. For females only 4000 < LD50 < 5000 mg/kg bw. For males only LD50 > 5000 mg/kg bw. In this hamster study, the doses administered were less toxic, leading to deaths of 1/5 male and 3/5 female hamsters at 5000 mg/kg bw.

Study 3 (Anon., (1981), Rabbit)

The acute oral LD_{50} was found to be 971 mg/kg bw. In this rabbit study, the doses administered were toxic leading to deaths in 3/3 male and female rabbits at 2000 mg/kg bw, and 2/3 male and female rabbits at 1000 mg/kg bw.

Study 4 (*Anon., (1980),* Mouse)

The acute oral LD_{50} was found to be 2444 mg/kg bw. In this mouse study, deaths were reported in 5/5 male and female mice at 5000 mg/kg bw, 4/5 male and 5/5 female mice at 3000 mg/kg bw, and 0/5 male and female mice at 2000 mg/kg bw.

Dermal route

The DS does not propose classification for penconazole (ISO) 1-[2-(2,4-dichlorophenyl)pentyl]-1H-1,2,4-triazole with respect to acute dermal toxicity. This is in line with the harmonised classification registered in ATP 006 and previously agreed by RAC in the final opinion document of June 2012 (following RAC-21). RAC does not propose a change to the original conclusion.

There was one acute dermal study with penconazole available, the guideline was not reported because the study predates the OECD guideline 402 of 1981. In this rat study the acute dermal LD_{50} was found to be > 3000 mg/kg, the administered doses of the test substance did not lead to deaths in any of the dose groups.

Inhalation route

The DS does not propose classification for penconazole (ISO) 1-[2-(2,4-dichlorophenyl)pentyl]-1H-1,2,4-triazole with respect to acute inhalation toxicity. There was one acute inhalation study with penconazole available, the guideline followed was OECD guideline TG433 (2018). In this rat study there were a number of deviations reported which did not affect the integrity of the study. The study is considered acceptable. The MMAD for Penconazole was 3.5 – 5.4 (mean: 4.4, aerosol). No animal deaths were recorded upon exposure to the highest achievable penconazole dose of 4046 mg/m^3, the acute rat inhalation LC_{50} (dust, nose only) is > 4.046 mg/L air/4h.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

Acute Oral toxicity

To be classified with acute toxicity Category 4 (oral), the lowest Category for this endpoint, the LD50 must fall between the following range: 300 < LD50 ≤ 2000 mg/kg bw. Toxicity was noted in the rat and rabbit studies leading to deaths.

The rabbit study (*Anon., 1981*) is considered the key study for setting the LD_{50}. The test substance was administered in 2% carboxymethyl-cellulose at 600, 1000 and 2000 mg/kg bw, respectively to three males and three females at each dose. No deaths were observed at the lowest dose of 600 mg/kg bw. Deaths were observed at 1000 mg/kg (66%) and 2000 mg/kg (100%). Non-linear regression and other statistical procedures calculate LD50 values at < 1000 mg/kg bw.

The acute oral LD_{50} in the rabbit study was found to be 971 mg/kg bw. Therefore, RAC concluded that a classification as **Acute Tox 4; H302** (harmful if swallowed) with an **Oral ATE value of 970 mg/kg bw** (rounded down to 2 significant figures) is warranted .

Acute Dermal toxicity

To be classified with acute toxicity Category 4 (dermal), the LD_{50} should be between 1000 < LD_{50} ≤ 2000 mg/kg bw. The acute dermal LD_{50} for penconazole in rat is > 3000 mg/kg bw, thus, no classification is warranted according to CLP criteria. RAC concluded that **no classification** is warranted for acute dermal toxicity.

Acute Inhalation toxicity

To be classified with acute toxicity Category 4 (Inhalation), the LC50 should lie between 1.0 < LC50 ≤ 5.0 mg/L (dusts and mists). The acute rat inhalation LC_{50} (dust, nose only) was > 4.046 mg/L air/4h (highest achievable concentration) and penconazole thus does not fulfil the CLP classification criteria for inhalation toxicity.

Overall, RAC concluded that **no classification** is warranted for acute inhalation toxicity.

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

Summary of the Dossier Submitter's proposal

Penconazole was investigated in several acute studies by the oral, dermal and inhalation routes. Insufficient detail was presented in the CLH report, the DS only commented on the inhalation toxicity study, the following is summarised from the DAR.

Rat acute oral study (*Anon., 1980*): common clinical signs occurred at doses ≥ 1000 mg/kg bw and included sedation, dyspnoea, curved or lateral/ventral body position, ruffled fur, and diarrhoea. There was no description as to the severity of the effects. All signs resolved by days 7-10 post exposure. Necropsy was uneventful.

Hamster acute oral study (*Anon., 1980*): common clinical signs occurred at doses ≥ 2000 mg/kg bw and included sedation, dyspnoea, curved or lateral/ventral body position, ruffled fur, diarrhoea, and exophthalmos. There was no description as to the severity of the effects. Salivation was seen after 3 to 5 hours of dosing at 4000 and 5000 mg/kg bw. Recovery of survivors occurred within 7 to 9 days. Necropsy was uneventful.

Rabbit acute oral study (*Anon., 1981*): Rabbits administered a dose of 600 mg/kg bw showed slight symptoms (slight ataxia, ruffled fur) after 2-3 hours post dosing only and thereafter remained free of symptoms. At higher doses (1000 and 2000 mg/kg bw) the most common clinical signs consisted of sedation, dyspnoea, curved body or prone on side, ataxia, and dacryorrhoea. There was no description as to the severity of the effects. Surviving rabbits of the 1000 mg/kg bw group recovered within 1 to 7 days post dose. Necropsy revealed partly congested organs. No further details provided.

Mouse acute oral study (*Anon., 1980*): Mice administered a dose of ≥ 1500 mg/kg bw showed sedation, dyspnoea, ruffled fur, and curved or ventral/lateral body position (no detail regarding severity). Recovery was complete in survivors of all groups by day 8 post-dosing. Necropsy was uneventful.

Rat acute dermal study (*Anon., 1980*): common clinical signs occurred at doses ≥ 2000 mg/kg bw and included dyspnoea, ruffled fur, and curved body position. There was no description as to the severity of the effects. All signs resolved by day 8 post exposure. Necropsy was uneventful.

The DS described clinical signs (slight to moderate sedation (at the 4 h time point only), moderate to severe dyspnea, curved body position and ruffled fur), observed in all animals exposed to penconazole in the inhalation study. These clinical signs were noted in both the controls and the test animals, though generally slightly more severe in the latter, and fully reversed by day 7 (test group). Necropsy was uneventful in the test animals.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance, which are not covered by the other hazard classes. STOT SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality. These criteria have not been met with penconazole**.**

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following a single exposure support classification into STOT SE1. No such evidence is available for Penconazole. There is no human data and animal studies do not support severe or significant effects at low dose levels.

Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Clinical signs of significance were noted but were not described as severe though there was also a lack of detail concerning the degree of the clinical signs observed. In addition, clinical signs were observed at doses close or exceeding those leading to mortality and in general there were no relevant findings at necropsy though partly congested organs were noted in the rabbit oral acute study at > LD_{50}.

For acute oral toxicity, four studies were performed on rat, mice, rabbit and hamsters. Penconazole was found to be most toxic by the oral route as lethality was noted in all species. Clinical signs consisted of sedation, dyspnoea, curved or lateral/ventral body position, ruffled fur, and diarrhoea. They were observed from one hour after dosing and persisted for up to 9 days, Gross pathology generally did not show any particular findings in any organ or tissue at necropsy, neither in decedents nor in surviving animals. One rat study was submitted for acute dermal toxicity with doses tested at 0, 2000, 2500, 3000 mg/kg bw for 24 hours. There were no animal deaths recorded up to the highest achievable dose of 3000 mg/kg bw. The most common clinical symptoms were dyspnoea, ruffled fur, and curved body position. All symptoms were reversible within 8 days after treatment.

With regard the STOT SE 3 specific criteria apply when classifying based on narcotic effects and respiratory tract irritation. In the dermal and oral toxicity studies, the clinical effects were observed at or above the LD_{50} which amounts to general toxicity. In the acute rat inhalation study, the LC_{50} (dust, nose only) was determined to be greater than the top dose achieved of 4.046 mg/L air/4h; no classification for acute inhalation was required. There were no animal deaths, symptoms included slight to moderate sedation, moderate to severe dyspnoea, curved body position and ruffled fur, which were observed in all animals at the end of the 4 h inhalation exposure and thereafter. Clinical signs were of a slightly more severe grade than in the vehicle control group and lasted 2 days longer. All rats had recovered completely on day 5 (vehicle control group) and on day 7 post-exposure (test group), respectively. While sedation effects were observed in several acute toxicity studies and could potentially support an additional classification for narcotic effects with STOT SE 3 – H336, sufficient details and evidence, e.g., on severity and duration of effects were not available to support the criteria for classification.

There is currently no harmonised classification for the Category of STOT SE. RAC concluded that **no classification** is warranted for STOT-SE (Category 1, 2 or 3).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS described a report on the Skin Irritation in the Rabbit after Single Application of Penconazole (Technical CGA71818). The study was conducted in 1980, prior to GLP and OECD TG 404, but followed EPA 163.81-5 (1978). There are some deviations from the current OECD 404 guideline (2015), e.g., exposure time was 24 hours instead of 4 hours; however, this is

considered a worst-case as compared to a 4 h exposure period. Despite the deviations, the study is considered acceptable.

In the study, skin irritation was investigated in groups of 3 male and 3 female New Zealand White rabbits after 24 h exposure to 0.5 g of penconazole tech. (88.4%) (penconazole concentration 50% in vehicle 70:30 v/v propylene glycol + saline). The DS does not describe the treatment procedure, however from the discussion of the results it can be deduced that test material was applied to intact and scarified skin. The only dermal effects observed were slight erythema of treated skin on both intact and scarified skin areas in all animals at patch removal (time 0 h). The mean scores for erythema or oedema at 24, 48 and 72 hours were zero.

Comments received during consultation

One comment was received from an MSCA, agreeing that, as the observed effects were below the threshold for classification as a skin irritant, no classification is required.

Assessment and comparison with the classification criteria

A single skin irritation study is available for Penconazole. Although the study is non-GLP and does not follow an OECD Test Guideline, RAC agrees with the DS that the study can be considered to assess classification for skin corrosion/irritation. According to CLP Regulation (EC) No. 1272/2008, substances shall be allocated to one of two categories within this hazard class: Category 1 (skin corrosion) and Category 2 (skin irritation). The major criterion for the irritation category is that at least 2 of 3 tested animals have a mean score of ≥ 2.3 and ≤ 4.0. In the presented study, the mean scores for erythema or oedema at 24, 48 and 72 hours were zero and no other signs of irritation were recorded. The criteria for Category 2 classification are clearly not met. Therefore, RAC concluded that **no classification** is warranted for skin corrosion/irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

A single eye irritation study in carried out in rabbits was available for penconazole. The study was conducted in 1988, according to GLP, but there are some deviations from the current OECD 405 guideline (2020). Despite these deviations, the study is considered acceptable. Three male and female rabbits were exposed to 100 mg of penconazole tech. Examination of the eyes for corneal opacity, iris lesions and conjunctiva redness and chemosis showed slight ocular irritation. Mean scores (24-72 h) were:

Corneal opacity: M: 0-0-0.67 F: 0-0-0.33;
Iris lesions: M: 0.33-0.67-0.33 F: 0.33-0.33-0.33;
Conjunctivae redness: M: 1.0-1.0-1.0 F: 1.0-1.0-1.0;
Conjunctivae chemosis: M: 0.67-1.0-0.67 F: 0.67-0.67-1.0.
Recovery was complete after 10 days.

Comments received during consultation

One MSCA commented, and stated effects observed in the available eye irritation study were below the trigger for classification as an eye irritant. They agreed with the proposal that classification for serious eye damage/eye irritation was not required for penconazole.

Assessment and comparison with the classification criteria

Penconazole does not satisfy the criteria for classification according to CLP requirement for serious eye damage (Category 1) or for eye irritation (Category 2) based on this study. Therefore, RAC concluded that **no classification** is warranted for serious eye damage/irritation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

A single skin sensitization study is available for penconazole. The study was conducted in 20 treatment and 10 control Albino Himalayan Spotted (GOHI) Guinea Pigs, using Penconazole technical (purity 96%). The study was a Maximisation Test following OECD 406, however the GLP-status of the study was not reported by the DS.

In a pre-test for dose selection, penconazole was administered by intradermal injection at doses of 0.5, 1.0, 3.0, and 5.0% and by topical administration at doses of 10, 20, 30, and 50% penconazole tech. The intradermal injections produced erythema and oedema (grade 1) at concentrations of 0.5, 1.0, 3.0 and 5.0%. Epidermal application produced erythema only at concentrations of 30% and 50%, and no signs of irritation at 10 or 20%. Based on these results, 5.0% penconazole technical in peanut oil was used for intradermal induction, 50% penconazole technical in Vaseline was used for epidermal induction and 20% penconazole technical in Vaseline was used for epidermal challenge.

In the main test, positive dermal skin reactions were observed on all animals following epidermal induction, but not in the vehicle controls. After challenge application, erythema was evident at the application site in 2/20 animals at 24 h and in 3/20 animals at 48h. Oedema was evident at the application site in 1/20 animals at 24 h and in 1/20 animals at 48 h. There were no skin responses among the vehicle control group. Erythema and oedema were examined and graded according to the Draize scoring scale, however the grades have not been reported by the DS. Based on these observed erythema and oedema in 3 animals, the sensitisation rate of penconazole in this maximisation test system was 15%. Overall, this Maximisation Test was negative for skin sensitisation.

Comments received during consultation

A comment was submitted from one MSCA, agreeing that skin sensitisation is not warranted based on the sensitisation rate observed in the available GPMT Study.

Assessment and comparison with the classification criteria

The CLP Regulation (EC) No. 1272/2008 allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B. Allocation into a subcategory can only occur when data are sufficient – if Category 1A cannot be excluded,

Category 1 should be applied instead of Category 1B. Criteria for classification based on data from animal studies is determined based on type of assay and concentration of substance at induction.

In this case of a Maximisation Test using a 5% intradermal induction dose, a response rate of ≥ 30 % responding would require classification. The observed response rate was 15%, therefore the criteria for classification are not met. Therefore, RAC concluded that **no classification** is warranted for skin sensitisation.

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

Summary of the Dossier Submitter's proposal

For the purposes of the CLH report, the DS summarised several studies associated with short term repeated dose toxicity of penconazole in different animal species including rat, dog, mice and rabbit. Two 28-day (gavage) and three 90-day (dietary) rat studies, two 90-day (dietary) studies in mice and a combined 90-day/1-year feeding study in beagle dogs were summarised. Additionally, a 21-day repeated dose dermal study in rabbits was also summarised. All studies have previously been evaluated by RAC in 2012. However, the DS questioned whether the observed single cases with liver effects such as necrosis and fibrosis in dogs should be considered as isolated cases. While awaiting the outcome of further discussions, the DS proposed that classification and labelling for STOT RE 2 H373 (liver) is warranted according to Regulation (EC) No. 1272/2008.

There were no new studies submitted for consideration in this hazard category. Given that penconazole has already been considered by RAC in 2012, the summary of the available studies provided in the previous RAC opinion are included in the additional key elements section below for completeness. The present evaluation by RAC reassesses the data and, while not contradicting the majority of what was reported in the final RAC opinion of 2012, it is important to note that the liver effects noted in both the dog 90-day and 1-year oral studies precludes a definitive acceptance of the liver effects being isolated incidences and warrants consideration of STOT RE 2.

In summary, the main topic for discussion in the present review of penconazole was that of hepatic necrosis and hepatic fibrosis in the dog where the DS proposed STOT RE 2 (H373 liver).

Comments received during consultation

Comment from one MS

There was one comment from the competent authority of one MS and one from the applicant during the public consultation. The MSCA discussed in-depth, the occurrence of hepatic necrosis (90-day study) and hepatic fibrosis (1-year study) in the dog.

Overall, the MSCA acknowledged that there was no new data provided on this human health endpoint and that RAC had already evaluated the data in 2012 and had concluded that classification for STOT RE was not required even though hepatotoxicity had been observed. Since the DS had requested re-discussion of the effects, the MSCA submitted their comment to determine if the observed cases of fibrosis in dogs should be considered as isolated cases.

The MSCA provided a discussion surrounding this issue in the dogs. The MSCA combined the incidences of liver fibrosis across all sacrificed animals for both sexes after 52 and 56 weeks. The

indication by DE was that the effects of inflammation with fibrosis in the liver were not isolated and occurred in 25% of the animals (17 mg/kg bw/day). However, the MSCA noted that they were in doubt as to whether the finding was considered "significant toxic effect" and reiterated that according to the CLP Guidance *„'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant"*. The MSCA also stated that they could not find significant functional disturbances supported by findings in biochemistry or haematology parameters at the mid dose.

90-day study, Beagle dog	**Males**				**Females**			
Penconazole (ppm)	0	100	500	5000	0	100	500	5000
Penconazole (mg/kg bw/d)	0	3.4	18.2	132	0	3.8	19.4	137
Body weight gain week -1 to 13° (%)	+27.5	+19.9	+19.1	-12.0	+29.0	+30.0	+21.3	-8.9%
Liver weight absolute (% change ctr)	-	+4.3	+20	+30	-	+15	+15	+22
Liver weight relative (% change ctr)	-	+1.6	+15	+75^{+}	-	+7.8	+24	+88^{+}
ALP (% change ctr)	-	+4	+7	+390*	-	-14	+1	+366*
GGT (% change ctr)	-	+14	+3	+1800	-	+0	+18	+932*
OCT (% change ctr)	-	-18	-18	+418*	-	+0	+20*	+480*
AST (% change ctr)	-	+0	+25	+154*		-7	+3	+143*
ALT (% change ctr)	-	-14	-8	+790*		-8	+5	+808*
Cytoplasmic vacuolisation	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
Inflammatory cell infiltration	0/4	0/4	1/4	4/4	0/4	0/4	0/4	4/4
Hepatocyte necrosis	0/4	0/4	1/4	4/4	0/4	0/4	0/4	4/4

°body weight gain in % of body weight prior to treatment (week -1)

* p 0.05, significant difference to control

$^{+}$ statistically significant positive trend from control to highest dose group

***Figure**: Summary of clinical chemistry finding in dogs as presented by the MSCA*

1-year dog study with Penconazole (1984)
"Inflammation with fibrosis" in the liver: Overview on incidences

Test item intake (ppm)		0	100	500	2500/5000
Males (mg/kg bw/d)		0	3.0	16.8	108
Females (mg/kg bw/d)		0	3.2	16.5	110
Males					
	Week 52	0/4	0/4	2/4	4/4
	Week 56	1/2	1/2	0/2	1/2
	Week 52+56	1/6	1/6	2/6	5/6
Females					
	Week 52	0/4	0/4	1/4	4/4
	Week 56	0/2	0/2	0/2	1/2
	Week 52+56	0/6	0/6	1/6	5/6
Males+Females					
	Week 52	0/8	0/8	3/8	8/8
	Week 56	1/4	1/4	0/4	2/4
	Weeks 52+56	1/12	1/12	3/12	10/12
Males					
	Week 52	0%	0%	50%	100%
	Week 56	50%	50%	0%	50%
	Week 52+56	**17%**	**17%**	**33%**	**83%**
Females					
	Week 52	0%	0%	25%	100%
	Week 56	0%	0%	0%	50%
	Week 52+56	**0%**	**0%**	**17%**	**83%**
Males+Females					
	Week 52	0%	0%	37.5%	100%
	Week 56	25%	25%	0%	50%
	Weeks 52+56	**8%**	**8%**	**25%**	**83%**

***Figure**: Summary of inflammation with fibrosis as presented by the MSCA*

Regarding hepatic necrosis, it was noted that only one animal displayed hepatic necrosis at the mid dose. Requesting clarification on the characterisation of its intensity; the DS indicated that all dogs at the high dose and one male of the mid dose group showed minimal, multifocal changes in the liver in the form of monocellular hepatocyte necrosis associated with minimal inflammatory cell infiltration. The DS also clarified that no historical control data on liver necrosis in beagle dogs was available. In addition, it was also noted by the DS that the individual raw-data from the 1-year study results did not contain information on whether the fibrosis in dogs was multi-focal or diffuse.

Comment from the applicant

A further comment was received from the applicant regarding the findings in the 90-day/1-year combined study. There was no significant or substantial increase in liver enzymes (ALT, ALP, AST, GGT) nor in the bilirubin levels in the male dog with minimal, multifocal liver necrosis at 500 ppm. The applicant stated that the occurrence of an isolated incidence of liver necrosis in this animal had no effect on liver function. Three dogs at 500 ppm (equivalent to 16.5-16.8 mg/kg bw/day) displayed minimal liver fibrosis and inflammation. The applicant considers these

findings to be incidental because the severity grading is low (minimal), the finding was only located in the peripheral lobular region of the liver and was not a diffuse liver finding. The applicant also noted that hepatic necrosis occurred in one male rat in the 1000 ppm group of the 1987b study and considered it incidental and not relevant for human health hazard assessment. The applicant did not consider the findings (of necrosis and fibrosis) in dogs sufficient to warrant a STOT RE classification.

Assessment and comparison with the classification criteria

During the previous RAC review of penconazole 2012 (see Additional key elements in the Appendix), it was concluded that "*The reported liver changes can be considered as only adaptive responses to the increased metabolic load. Although some liver changes at 16.9/16.7 (M/F) mg/kg bw/day (500 ppm) in dog studies could be considered as severe, they appear as isolated cases: necrosis in 1 male out of 4 in the 90-day study and also fibrosis in 1 male out of 4 when the study was prolonged to 1-year. A similar interpretation can be made for the hepatic degeneration observed in one rat 90-day study at 72 mg/kg bw/day (1000 ppm). Although the effective dose levels in both dogs and rats are within the 10 < C ⩽ 100 mg / kg body weight/day range, RAC's conclusion is that a classification for specific target organ toxicity is not required under Regulation (EC) 1272/2008 or Directive 67/548/EEC*".

Classification criteria

According to CLP Regulation 1272/2008 *"STOT RE is assigned based on findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health".*

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6).

RAC conclusion

There was one key report (Anon., 1984) relied upon by the DS and served as the basis for the proposed classification of STOT RE 2. This report consisted of two oral dietary dog studies; a 90-day and 1-year with either 4 or 6 animals/sex/dose. In both studies the mid dose group and top dose group interval was noted to cross the guidance value (GV) for STOT RE 2.

RAC recognises that the liver is the target organ for penconazole and supported by evidence in the rat and mice 90-day oral studies. There appears to be a steep dose response between the mid to top dose intervals and it is further noted that the top dose group in each case is not so far removed from the GV or adjusted GV for Category 2 classification. Under these circumstances CLP guidance also allows for classification based on effects seen above the GV for STOT RE 2 (Annex I: 3.9.2.9.9).

Following 90 days of treatment in the dog, liver effects were seen at ≥ 500ppm (equivalent to 18.2-19.4 mg/kg bw/day – which is within the 90-day guidance value range of 10-100 mg/kg bw/day for STOT RE Cat 2). Liver effects that were considered potentially severe enough to meet the criteria for STOT RE 2 classification included multifocal liver necrosis seen in only one dog at the mid dose along with large increases in liver weight (both absolute and relative to bodyweight, 15-24% above controls). At the top dose however (132-137 mg/kg bw/day), severe effects were noted on liver weight, hepatic necrosis in all animals, male and female, along with drastic increases in several key liver enzymes indicative of grave liver insult. Gamma-glutamyl transferase (GGT) is common to several tissues but is also a key marker in liver function tests. Serum levels increase markedly with bile duct damage, and it is recognised as a marker of cholestasis. Serum alanine transaminase (ALT) levels can also be specific for hepatocellular injury, due to loss of hepatocyte membrane integrity or necrosis, particularly when the magnitude of the ALT increase is greater than that of AST which is the case here in both dog studies at the top dose. Ornithine transcarbamoylase (OCT) is a key enzyme in the urea cycle, catalysing the reaction of ornithine with carbamoyl phosphate to form citrulline and is almost exclusively located in the mitochondria of hepatocytes. Raised serum levels are associated with hepatocyte and mitochondrial damage.

90-day study, Beagle dog	**Males**				**Females**			
Penconazole (ppm)	0	100	500	5000	0	100	500	5000
Penconazole (mg/kg bw/d)	0	3.4	18.2	132	0	3.8	19.4	137
Body weight gain week -1 to 13° (%)	+27.5	+19.9	+19.1	-12.0	+29.0	+30.0	+21.3	-8.9%
Liver weight absolute (% change ctr)	-	+4.3	+20	+30	-	+15	+15	+22
Liver weight relative (% change ctr)	-	+1.6	+15	+75^{+}	-	+7.8	+24	+88^{+}
ALP (% change ctr)	-	+4	+7	+390*	-	-14	+1	+366*
GGT (% change ctr)	-	+14	+3	+1800	-	+0	+18	+932*
OCT (% change ctr)	-	-18	-18	+418*	-	+0	+20*	+480*
AST (% change ctr)	-	+0	+25	+154*		-7	+3	+143*
ALT (% change ctr)	-	-14	-8	+790*		-8	+5	+808*
Cytoplasmic vacuolisation	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
Inflammatory cell infiltration	0/4	0/4	1/4	4/4	0/4	0/4	0/4	4/4
Hepatocyte necrosis	0/4	0/4	1/4	4/4	0/4	0/4	0/4	4/4

°body weight gain in % of body weight prior to treatment (week -1)

* p 0.05, significant difference to control

$^{+}$ statistically significant positive trend from control to highest dose group

Cytoplasmic vacuolisation was also noted in top dose males and has been recorded as a common response in xenobiotic stresses animals. It can also be an early finding and indicative of swelling of organelles, perturbations in lipid deposition or glycogen storage. Sudan III (detection of lipid

droplets) and PAS (glycogen stain) negative liver sections indicated no lipid build-up but also did not confirm if the cytoplasmic vacuolation was due to glycogen build-up (considered a protective response by some authors[2]). In the 90-day rat study (1987b), cytoplasmic vacuolation was not associated with Oil Red O staining indicating no lipid build-up and presumed to not be degenerative in nature.

Following 1 year of treatment, liver effects were also seen at ≥ 500 ppm (equivalent to 16.5-16.8 mg/kg bw/day – which is within the 1-year guidance value range of 2.5-25 mg/kg bw/day for STOT RE Cat 2). Liver effects considered potentially severe enough to meet the criteria for STOT RE 2 classification at the mid dose included liver fibrosis seen in three dogs (2/6 males, 1/6 females) and large increases in liver weight in females. Similar to the responses noted in the 90-day study, top dose animals (108-110 mg/kg bw/day) exhibited severe effects on liver weight, hepatic necrosis, inflammation with fibrosis (10/12 animals, male and females combined), along with drastic increases in several liver enzymes (especially OCT), indicative of grave liver insult.

1-yr study, Beagle dog	**Males**				**Females**			
Pencoazole (ppm)	0	100	500	2500°	0	100	500	2500
Penconazole (mg/kg bw/d)	0	3.0	16.8	108	0	3.2	16.5	110
Liver weight absolute (%)		-14	-10	+27	-	+6.3	+27	+46*
Liver weight relative (%)		-24	-6.8	+35	-	+3.7	+28	+63*
ALP (% change rel. to ctrl)	-	-1.5	+60	+425*	-	-9.0	-18	+381*
GGT (%)	-	-	+41	+504*	-	+13	-22	+313*
OCT (%)	-	-7.5	-6.0	+1273*	-	-8.5	+23	+1700*
AST (%)	-	+14	+8.6	+157	-	-	+13	+109*
ALT (%)	-	+18	+8.2	+454*	-	-7.0	+2.4	+683*
Cytoplasmic vacuolisation^{+}	0/6	0/6	0/6	2/6	0/6	0/6	0/6	2/6
Inflammatory cell infiltration^{+}	1/6	2/6	2/6	1/6	1/6	2/6	1/6	0/6
Inflammation with fibrosis^{+}	1/6	1/6	2/6	5/6	0/6	0/6	1/6	5/6
Hepatocyte necrosis^{+}	0/6	0/6	0/6	2/6	0/6	0/6	0/6	2/6

RAC notes there was no detailed information on the magnitude of the adversity in the observed liver effects. The studies are old and reporting of effects was not ideal. The data clearly show however, that the top doses of penconazole result in substantial adverse effects close to the upper GV for STOT RE 2. The adverse effects on liver weight and enzyme levels reverse upon recovery after cessation of dosing in dogs.

The liver effects under consideration here occurred at an exposure level relevant for STOT RE Category 2 consideration. It is noted by RAC that the dose spacing was suboptimal and that classification may have been more robustly supported if an appropriate dosing interval with a slightly higher dose level greater than the mid dose employed was tested (but below the GV cut-offs for Category 2). There appears to be a clear dose response relationship in the liver to Penconazole. The cases of necrosis and fibrosis seen in the dog studies at a dose less than the GV are therefore not considered to be incidental. A severe impact on the liver at doses marginally greater than the GV illustrate the hepatotoxicity of penconazole. Effects noted at values above the GV may also be used to substantiate a proposal for STOT RE 2 as outlined in the CLP guidance:

2 *Nayak et al.* The nature and significance of liver cell vacuolation following hepatocellular injury - an analysis based on observations on rats rendered tolerant to hepatotoxic damage. Virchows Arch. 1996 Aug;428(6):353-65.

Annex I: 3.9.2.9.9. *"… a specific profile of toxicity may be seen in animal studies occurring at or above a guidance value, such as ⩾ 100 mg/kg bw/day by the oral route, … in view of the weight of evidence, classification is the prudent action to take."*

RAC agrees with the DS and the applicant that the liver is a target organ for Penconazole. RAC further notes that the DS did not submit any new data to elucidate the findings of hepatic necrosis and fibrosis, there was no new evidence to further support the proposal for STOT RE 2 (H373 Liver) classification. RAC agrees that the liver is clearly the primary target organ, with the dog being the most sensitive species. The observed findings of necrosis and inflammation with fibrosis were observed in both the mid and high dose groups, where severe toxicity was additionally observed just outside the GV for Category 2 classification, indicating a steep dose response curve for these liver effects. RAC identified convincing evidence of significant functional disturbances related to liver toxicity reflected in large changes in key enzymes at the top dose (particularly in the 90-day study close to the GV). RAC also acknowledges that hepatic fibrosis occurred in a relatively low number of animals (in addition to an animal in the control group and one animal in the low dose group) at a dose principally relevant for STOT RE 2 classification, (i.e. 17 mg/kg bw/day). RAC also notes supportive data from the rat 90-day study (91987b), where relevant liver effects were seen at 72 mg/kg bw/day in males:

- single (1/15) incidence of hepatocellular degeneration (no description of severity) [5/15 at next dose].
- 12/15 incidences of hepatocellular hypertrophy [15/15 at next dose].
- 4/15 incidences of hepatocytic vacuolisation [11/15 at top dose, 179 mg/kg bw/day].

RAC concludes that the effects seen in the dogs are considered biologically significant, and are sufficient to meet the criteria for classification for STOT RE 2 as proposed by the DS. Therefore, RAC concluded that penconazole warrants a classification for **STOT RE 2; H373 (liver).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that penconazole was tested in a range of GLP and OECD guideline compliant *in vitro* and *in vivo* genotoxicity assays, details were supplied in table 39 of the CLH report.

In vitro assays included:

- 4 × *in vitro* Ames tests (reverse mutation assay with *Salmonella typhimurium* and/or *Escherichia coli*) according to OECD 471, performed 1984 - 2010; different batches of technical material - all negative.
- 1 × *in vitro* mammalian cell gene mutation test (HPRT assay, Chinese hamster V79 cells,) OECD 476, performed 1999; tested in preliminary cytotoxicity and mutagenicity assay using technical material (EN603012) at varying concentrations – negative.
- 1 × *in vitro* mammalian chromosome aberration test (Chinese hamster ovary (CHO) cells) according to guideline OECD 473, performed 1999; tested using technical material (EN603012), between 0.78 and 100 µg/ml; 200 metaphases tested (recommended 2000) – negative.
- 1 × *in vitro* unscheduled DNA synthesis in mammalian cells (DNA Damage and Repair) OECD 482, Primary hepatocytes toxicity tests between 5 to 320 µg/ml (technical material P.11-14), performed 1984, using primary rat hepatocytes – negative.

In vivo assays included:

- 1 × mouse micronucleus tests, OECD 474, single oral dose (gavage) at MTD: 200-800 mg/kg bw (M) and 125-500 mg/kg bw (F), performed 1999 with penconazole tech (EN603012) - negative. However, it is noted in the CLH reported that blood samples were not taken at appropriate times which failed to demonstrated exposure of the bone marrow occurred. Therefore, RAC agrees that this study may be considered supplementary.

Conclusion

According to the CLH report, no conclusion on mutagenicity was possible by the DS. The DS requested a re-analysis of the *in vivo* study indicating that it could possibly provide a better basis to draw a conclusion.

Comments received during consultation

There was one comment received from a MSCA agreeing that the findings observed in both the *in vitro* and *in vivo* studies do not indicate relevant genotoxic potential. Clarification was sought by the DS regarding the available confidential *in vitro* micronucleus test to conclude on the genotoxic potential of penconazole. The DS confirmed that this *in vitro* test can support the *in vivo* result on clastogenicity and aneugenicity. While the *in vivo* MN test was negative it did not meet the acceptability criteria due to the low numbers of cells analysed. Classification for mutagenicity was not proposed by the MSCA.

RAC response

There are no new genotoxicity data to support classification of Penconazole. In this review of Penconazole, RAC is broadly in agreement with the previous RAC opinion of 2012. The lack of support for classification for genotoxicity was also noted by one MSCA during the public commenting period.

RAC notes that while some of the studies were of low reliability and supplementary, there are negative results overall to lower the concern for genotoxicity. The existing data, however, according to the present assessment, is not sufficiently robust to eradicate all concerns and the proposal for no classification is based on inconclusive data in this case.

Assessment and comparison with the classification criteria

The available *in vitro* assay on chromosome aberration is of limited reliability (due to the low number of scored cells) and because of the statistically significant increase over the concurrent negative control in one test concentration in one experiment. The overall result is not clear when strictly evaluated according to OECD TG 473 (2016). The available *in vivo* micronucleus test in mice is also of limited reliability because of insufficient numbers of scored cells. The *in vitro* micronucleus test confirms the absence of both aneugenic and clastogenic potential for penconazole and the negative result for clastogenicity in the supplementary chromosomal aberration assay.

Taking all of the available studies into consideration and the fact that many uncertainties remain, RAC concludes that no classification is warranted on germ cell mutagenicity, based on inconclusive data.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Three GLP and guideline compliant long-term oral (dietary) toxicity/carcinogenicity studies were available to the DS: an OECD 453 study in mice (*Anon., 1985*), an OECD 453 study in rats (*Anon., 1985a*) and an OECD 451 study in mice (*Anon., 2004*). The latter study (2004) was fully acceptable and reliable, however, the two older studies were considered supportive only, as the selected dose levels were too low to produce any significant toxicological effects or to approach the MTD. During the renewal of Penconazole (RAR, 2021), the RMS requested a statement from the applicant justifying the dose selection for the studies, which was received, and this was commented on in section B.6.5 of Volume 3 of the RAR (2021).

In the two supportive studies (*Anon., 1985 and 1985a*), there were no significant toxicological effects and no tumours at the highest dose level of 300 ppm. In the third study (*Anon., 2004*), a top dose of 1500 ppm produced no tumours, but body weight development was reduced and an increase in liver weight, associated with an increase in hepatocyte vacuolisation, was observed.

In the statement on dose-selection for the studies, the applicant outlined the results of two 90-day rat studies which were used to define the doses for the long-term rat study (*Anon., 1985a*). In the first study, doses of 30, 300 and 3000 ppm were tested and a NOAEL of 300 ppm was set based on reduction in body weight, increased liver weight and increased blood cholesterol. In the second, doses of 10, 30 and 100 ppm were tested, and the NOAEL was 100 ppm, based on absence of any treatment-related effects. They state that a decision was made to repeat a carcinogenicity study with higher doses, in recognition of the fact that the previous two studies had been performed at relatively low doses. Hence, the Anon., (2004) study was performed. The mouse was chosen as the test species because it was decided that the mouse liver is the critical target organ for penconazole with respect to tumorigenic potential and the dose levels were selected based on results of a new 90-day range finding study in mice (Anon., 2002).

The DS noted that EFSA (2008) previously concluded that penconazole had no carcinogenic potential and did not need to be tested at higher doses in rats and also that according to RAC (2012), the negative result of the 2004 study in mice (Anon., (2004), together with the supportive 1985 studies in mice (Anon., 1985) and rats (Anon., 1985a) indicated no carcinogenic potential for penconazole. The DS (and RMS) called into question whether these lifetime studies were sufficient to exclude a carcinogenic potential of penconazole and whether additional testing of long-term toxicity and carcinogenesis at higher doses in rats may be needed. The DS proposed a re-discussion of these studies, taking a holistic approach to the evaluation by considering other endpoints such as the genotoxic potential of penconazole. The DS did not indicate a clear position on the carcinogenicity endpoint.

Comments received during consultation

One comment was received from an MSCA during the public consultation. The MSCA considered the studies sufficient to conclude on classification but supported the proposal for a re-discussion, noting that further information has been requested for the purpose of the PPP assessment.

Assessment and comparison with the classification criteria

In the present case for penconazole, none of the three bioassays in animals recorded any increase in incidence of tumours or displayed any indication of a carcinogenic effect or pre-neoplastic changes. However, two of the studies (one rat, one mouse) were critically noted as having used doses below the MTD which did not produce any significant toxicity and were graded as supportive

from a regulatory point of view. It was previously concluded that the results of the newer mouse study was fully reliable (*Anon., 2004*), and combined with the two supportive studies (Anon., 1985 and 1985a) there was sufficient reason to draw a conclusion on carcinogenicity with no further studies at higher doses required (EFSA, 2008 and RAC, 2012). The absence of tumours and any evidence of carcinogenicity means the criteria for Category 1 or 2 classification are not met.

The DS argues that there should be further discussion on the sufficiency of these three studies to exclude a carcinogenic potential of Penconazole and to reach a conclusion on classification. They requested justification from the applicant on the dose-selection for the log-term toxicity and carcinogenesis studies, which was provided and is summarised above. RAC disagrees with the DS that any further evaluation will result in a clearer or more robust assessment of the existing data or that any further studies may be warranted. The RAR is quite extensive and well-reported, support for no classification is agreed by RAC though RAC acknowledges the deficiency in dosing and in this point (i.e., regarding uncertainty), fully supports the DS comments. It is outside the scope of RAC to recommend additional testing at higher doses. It is appropriate however, to point out the deficiencies in the available studies.

RAC notes the lack of genotoxic potential in penconazole. Further information on other endpoints not presented in the CLH report is not currently available. Read-across with other triazole substances has not been presented or assessed by the DS.

Overall, RAC agrees that the existing data supports no classification for carcinogenicity, reliant mainly on the *Anon., 2004* mouse study, the lack of appropriate dosing in the other two older studies does introduce some uncertainty but this cannot be regarded as inconclusive data. Therefore, RAC concludes that no classification is warranted for carcinogenicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter proposed classification as Repr. 2, H361d. This is in line with the outcome of the previous RAC evaluation (2012) and the current Annex VI entry.

Penconazole has been evaluated for reproductive toxicity in two multi-generational studies in rats and four developmental toxicity studies in rats and rabbits. Both multi-generational studies were non-OECD 416 compliant (one was GLP and the other non-GLP) and can only be considered as supplemental due to significant deficiencies. One of the rat developmental toxicity studies was only considered as supportive (but acceptable by the RMS in the RAR), having been conducted before the relevant OECD TG 414 was available. Two of the three remaining developmental toxicity studies were GLP and OECD TG 414 compliant and all three were considered acceptable by both the DS and RMS. The DS did not present any findings or analysis for effects on or via lactation. No conclusion on this endpoint was reported.

Adverse effects on sexual function and fertility

<u>Studies in the rat</u>

The two multi-generational studies (Anon., 1983 and Anon., 1987) may not have achieved sufficient dosing (they did not consistently produce > 10% reduced bodyweight development or

other signs to indicate that the MTD was achieved), hence can only be considered supportive. On the other hand, > 10% mortality was observed amongst dams post-partum in the high dose groups of both F0 and F1 generations (3/20 for F0 and F1 dams). Deviations from OECD 416 included (in one of both studies) using large dose intervals (dose intervals were 10-fold that of the previous dose), not continuing dosing for 10 weeks before mating period, a lack of evaluation of oestrous cycle length and sperm parameters, testis and epididymis were not weighed, anogenital distance was not measured, the number of corpora lutea was not determined, and not all organs were weighed or histopathologically examined at termination.

1) In the first study (*Anon., 1983*), penconazole (purity 91.7%) was administered orally in the diet to Tif:RAIf(SPF) rats (20/sex/group) at concentrations of 0, 80, 400, 2000 ppm. Treatment started for F0 and F1 animals when they were about 28-30 days old and continued for 62 days (about 9 weeks) prior to mating, during the 12-day mating period through to termination i.e., after the litters had weaned. Parental toxicity was seen at the top dose, presenting as a slightly reduced body weight gain and food consumption, which was most pronounced in females during the premating and gestation periods. An increase in dam mortality at/or shortly after parturition and during lactation was noted, as well as a slight delay in parturition and prolongation of the gestation period. Consequently, the reproductive NOAEL was set at 400 ppm (29.7 mg/kg bw/day in females).

2) In the second study (*Anon., 1987*, GLP compliant, EPA 83-4), penconazole (purity 98.7%) was administered orally in the diet to Sprague-Dawley rats (30/sex/dose) at concentrations of 0, 25, 250 and 2500 ppm. The duration of exposure was 63 days (9 weeks, F0) and 84 days (12 weeks, F1) prior to mating, during the maximum 3 weeks mating period through to termination, i.e., after the litters had weaned. Signs of generalised toxicity similar to the first study were seen at the top dose level. Gestation length was unaffected, and no dam mortality was observed. Consequently, the reproductive NOAEL was set at 250 ppm (22.7 mg/kg bw/day in females).

Adverse Effects on Development

Studies in the rat

Two developmental studies were conducted in rats. Both had several deviations from the current OECD guideline 414 (2018), the most severe being test substance administration to pregnant animals after implantation until GD15 in the case of the rats and GD18 in the case of rabbits, weight and histopathological assessment of the thyroid gland should have been taken, number of corpora lutea should have been determined, the anogenital distance should have been measured, and blood samples should have been collected to assess thyroid hormones and TSH.

1) In the first developmental toxicity study (*Anon., 1981*), groups of female Tif:RAIf (SPF) rats received penconazole (purity 88.4%) orally by gavage at concentrations of 0, 300 mg/kg bw/day for days 6-15 of gestation (preliminary test), 0, 30, 100, 300 mg/kg bw/day for days 6-15 of gestation (main test) or 0, 300, 450 mg/kg bw/day for days 10-14 of gestation (supplementary test). Unspecific maternal toxicity was shown by mortality, reduced body weight development and food consumption at the top dose groups of 300/450 mg/kg bw/day. Increases in post-implantation loss, partly exceeding available HCD, were seen. An increase in dead foetuses were seen at 450 mg/kg bw/day. Reduced foetal weights were reported. The overall number of skeletal anomalies was increased at 300 mg/kg bw/day (main

study only) and 450 mg/kg bw/day (supplementary study), however the type of abnormalities varied widely and were not reproducible between the main and supplementary studies. Consequently, the maternal and developmental NOAEL was set at 100 mg/kg bw/day.

2) In the second study (*Anon., 1985*), groups of female Sprague-Dawley rats received penconazole (purity 98.7%) orally by gavage at concentrations of 0, 5, 100, 500 mg/kg bw/day for days 6-15 of gestation. Originally, 750 mg/kg bw/day was chosen as the top dose, but this was reduced due to excessive toxicity. Unspecific maternal toxicity was again seen at the top dose, consisting of mortality, clinical signs and reduced body weight development and food consumption. As in the previous study, increases in post-implantation loss which partly exceeded the available HCD were reported, as were reduced foetal weights. Additionally, a reduced number of live foetuses per dam and an increased number of runt foetuses were reported. Skeletal abnormalities consisted of delayed ossification and increases in 14^{th} ribs. The maternal and developmental NOAEL was again set at 100 mg/kg bw/day.

Studies in the rabbit

Two developmental studies were conducted in rabbits. Both had several deviations from the current OECD TG 414 (2018), the most severe being administration of test substance after implantation up until several days prior to the day of scheduled humane killing, each test and control group should contain a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy, weight and histopathological assessment of the thyroid gland should be taken and brain, nasal passages and tongue should be examined from half of the foetuses.

1) In the first study (*Anon., 1982*), penconazole (purity 91.7%) was administered orally in the diet to female Chinchilla rabbits (20/dose) at concentrations of 0, 25, 75, 150 mg/kg bw/day (GD 6-18). Unspecific maternal toxicity was observed in the top dose group as reduced body weight development and food consumption. Developmental malformations (hydrocephalus and microphthalmia) were reported in the high dose group, with the former slightly exceeding the available HCD and the latter staying within the range of the HCD. Two foetuses had both microphthalmia and hydrocephalus. The maternal and developmental NOAEL was set at 75 mg/kg bw/d.

2) In the second study (*Anon., 1985*), penconazole (purity 98.7%) was administered to female New Zealand White rabbits in the diet at concentrations of 0, 10, 50, 200 mg/kg bw/day (GD 7-19). Unspecific maternal toxicity was again characterised by reduced body weight development and food consumption in the top dose group. Clinical signs were also reported. Developmental effects included post-implantation loss and a slightly reduced number of live foetuses per litter, although they did not exceed the HCD. Additionally, two dead foetuses were recorded. Foetuses with hyoid body and/or arches unossified and reduced ossification of the skull were also observed and exceeded available HCD ranges. The maternal and developmental NOAEL was set at 50 mg/kg bw/d.

Comments received during consultation

One comment was received from an MSCA during the public consultation. The MSCA followed a weight-of-evidence approach for evaluating classification. They pointed to the observed developmental effects, the current harmonised classification, and the fact that other triazoles are

known to cause developmental effects (and are classified as such), as sufficient evidence to support classification as Repr. 2, H361d.

Assessment and comparison with the classification criteria

Assessment of effects on sexual function and fertility

According to the CLP criteria, adverse effects on sexual function and fertility includes alterations to the female and male reproductive system, effects on the onset of puberty, fertility, parturition, pregnancy outcomes plus more. Effects on parturition and pregnancy outcomes were observed (inconsistently) across the various multi-generation and developmental toxicity studies, although attributing the effects to a clear, reproductive cause, is challenging.

Effects on the duration of pregnancy and survival at/post-parturition were observed in the first multi-generation study in rats (*Anon., 1983*). Mean duration of pregnancy significantly increased in the F0 generation from 21.1 days in the control group to 21.6 days in the high dose group, and in the F1 generation from 21.3 days in the control group to 21.8 days in the high dose group (not statistically significant). One F0 dam of the mid dose group died during delivery and 3 of the high dose group died post parturition (Days 0, 4, 11). Similarly, in the F1 generation, one dam of the mid dose group died (Day 0 p.p.), as did 3 from the high dose group (Days 2,2,4 p.p.), however one dam of the control group also died (Day 19 p.p.). The deaths at and shortly after parturition may reflect the prolonged parturition process, as does the reported increase in perinatal mortality in the offspring (mostly presenting as total litter loss) which are considered a consequence of dystocia rather than developmental toxicity. The relevance of the later deaths is not clear, but they are probably more likely related to maternal toxicity than to dystocia. In a second multi-generation study in the rat (*Anon., 1987*), in which higher doses and a test substance with higher purity were used, no effect on pregnancy duration or parturition was observed.

Similar inconsistencies were seen in the developmental studies in rats and rabbits, with effects being seen in one study but not being reproduced in second. In the first developmental study in rats (*Anon., 1981*), 2/25 dams in the high dose group died on GD 21 without other signs of toxicity. In a supplementary study to investigate this unusual finding, several dams (0/15, 4/15 and 2/15 dams at 0, 300, 450 mg/kg bw/day) died on GD 21. Whether these deaths are a true reproductive effect, or a result of generalised maternal toxicity, is difficult to ascertain. In all cases, deaths occurred up to 5 days after the end of treatment and about one day before natural parturition should have commenced and body weight gain was slightly decreased during treatment with 300 mg/kg bw in the main study and markedly decreased at 300 and 450 mg/kg bw/day in the supplementary study. In the second study (*Anon., 1985*), no such effects were seen, even at a maternally toxic dose of 500 mg/kg bw/d.

In one developmental study in rabbits (*Anon., 1985*), there was evidence of premature parturition in all treated groups. Several does delivered either 1 day prior to or on the day of the scheduled caesarean section (0/18, 2/16, 2/14, 1/18 at 0, 10, 50, 200 mg/kg bw/day, respectively), with a combined incidence of 10% compared to the 3% incidence in the HCD. All their foetuses were normal and necropsy findings did not indicate any treatment-related findings. No corresponding effects were seen up to doses of 150 mg/kg bw/day in a second developmental study in rabbits (*Anon., 1982*).

Repeated dose toxicity in dogs (Anon., 1984) indicated effects of concern in males. In the high dose groups there was a moderate to marked reduction in spermatogenic activity, characterised by atrophy of the seminiferous epithelium and associated with the formation of giant cells, and

the absence of spermatozoa in the epididymis (which contained cellular debris). The top dose was above the MTD for the first 19 weeks in the 1-year study before being reduced from 5000 to 2500 ppm. The findings in the testis and epididymis were considered by the RMS in the plant protection dossier to be secondary to bw effects (body weight loss) seen in the first 19 weeks of the 1-year study. The age of the studies, lack of support data from other repeat dose studies and low number of animals tested are not considered sufficient by RAC to warrant a classification (inconclusive data).

1-yr study, Beagle dog	**Males**			
Pencoazole (ppm)	0	100	500	2500°
Penconazole (mg/kg bw/d)	0	3.0	16.8	108
Testes weight absolute (%)	-	-4.3	-7.8	-15
Testes weight relative (%)	-	-14	-3.8	-8.8
Reduced spermatogenesis	0/6	0/6	0/6	2/6
Tubular atrophy	0/6	1/6	1/6	4/6
Epididymis: cellular debris	0/6	0/6	0/6	0/6

90-day study, Beagle dog	**Males**			
Pencoazole (ppm)	0	100	500	2500°
Penconazole (mg/kg bw/d)	0	3.0	16.8	108
Testes weight absolute (%)	-	+27	+0.2	-47
Testes weight relative (%)	-	+23	+4.4	-27
Reduced spermatogenesis	0/4	0/4	0/4	4/4
Tubular atrophy	0/4	0/4	0/4	0/4
Epididymis: cellular debris	0/4	0/4	0/4	4/4

Comparison with classification criteria – sexual function and fertility

There was no information on the potential of penconazole to adversely affect sexual function and fertility in humans and therefore classification in Category 1A is not warranted.
Classification in Category 1B (presumed human reproductive toxicant) should be largely based on data from animal studies that provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects. RAC concludes that the available studies do not provide robust, clear evidence of reproductive toxicity for fertility and or sexual function. In addition, effects on parturition and pregnancy were not always reproducible, consistent, or significantly above the HCD in either species (rat and rabbit).

Regarding classification in Category 2 (suspected human reproductive toxicant), RAC notes little consistency or confidence in the results of the studies to warrant a classification proposal. The inconsistency in results was observed between studies, between and within species and within the effects themselves. With respect to maternal deaths, death of dams was seen in a rat 2-generation study (*Anon., 1983*) and a developmental study (*Anon., 1981*) conducted by one laboratory in one strain of rats, but not in a 2-generation (*Anon., 1987*) and developmental study (*Anon., 1985*) with other strains of rats at slightly higher doses tested, nor in developmental studies with rabbits (*Anon., 1982; Anon., 1985*). There was inconsistency in the timing of deaths,

and those that occurred 2 days or more after parturition (*Anon., 1983*) were considered to be related to maternal toxicity, not dystocia. As to the duration of pregnancy, a slightly prolonged duration was seen in the rat 2-generation study by *Anon., 1983* (together with possible consequences for total litter loss and live litter size), but not in the rat 2-generation by *Anon., 1987* that was conducted with a slightly higher top dose. In rabbits on the other hand, premature parturition was seen, but only in one study (*Anon., 1985*), not in a second study with another strain (*Anon., 1982*). The relevance of the finding in the 1985 study is doubtful, given the absence of dose-response and all foetuses being normal.
Therefore, RAC concludes that no classification is warranted for effects on sexual function and fertility.

Assessment of effects on development

Several findings that were indicative of developmental toxicity were observed in the available developmental and multi-generation studies. According to the CLP criteria, developmental toxicity includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during pre-natal development, or postnatally, to the time of sexual maturation.

Increases in resorptions were observed in both rat and rabbit studies. The incidences of early resorption in the first developmental study in the rat (*Anon., 1981*), were 4.8%. 5.9%, 8.1%, 9.0% at 0, 30, 100, 300 mg/kg bw/day, although none were statistically significant and were within the historical control range except for the high dose group. In the second developmental study in the rat (*Anon., 1985*), the incidences of resorptions expressed as a % of implantations were 2.2%, 4.4%, 3.6% and 18.9% at 0, 5, 100 and 500 mg/kg bw/day, respectively. At the high dose the increase was statistically significant and was concomitant with considerable maternal toxicity (death of 2/25 dams, severely reduced body weight gain (-41%), clinical symptoms). An increase in post-implantation loss was also seen in one of the multi-generation studies (*Anon., 1987*) at the high dose of 2500 ppm. In rabbits, the incidences of early resorptions in the first rabbit developmental study (*Anon., 1982*) study were 4.8%, 6.0%, 0.9% and 9.7% at 0, 20, 75 and 150 mg/kg bw/day, respectively. At the high dose, maternal toxicity in the form of reduced body weight development was noted at different time points. In the 1985 rabbit study, the corresponding incidences were 6.6%, 12.5%, 1.4% and 16.4% at 0, 10, 50 and 200 mg/kg bw/day, respectively. It should be noted that in the case of the high-dose group, one of the females was responsible for one third of the cases of resorptions. Maternal toxicity was characterised by a 37% reduction in daily food consumption (GD 7-20, but particularly marked during the first week), which was associated with weight loss over the same period, such that there was almost no weight change throughout pregnancy. None of the findings in the rabbit studies was statistically significant and clear dose–response relationships were not apparent.

Structural abnormalities consisted of incomplete/absent skeletal ossification and various malformations. Incomplete/absent skeletal ossification was recorded in two rat and one rabbit developmental studies respectively (*Anon., 1981; Anon., 1985; Anon., 1985*). Such findings can be regarded as variations or delays in development and were only seen in the presence of maternal toxicity, such that classification may not be warranted. Supernumerary cervical ribs were reported in one rat study (*Anon., 1985*), but were also associated with maternal toxicity. Microphthalmia and hydrocephalus were observed in the first rabbit developmental study (*Anon., 1982*) at the high dose of 150 mg/kg bw/d. One foetus had bilateral microphthalmia alone, while two had bilateral microphthalmia in combination with internal hydrocephalus, giving incidences of 1.6% (foetal) and 12.5% (litter); these were just outside the historical control range. However, the combined incidence of microphthalmia in the three affected foetuses (2.4%) was still within

the range of the historical control data. These kinds of rare malformations are unlikely to be related to the maternal toxicity observed (changes in body weight).

Table B.6.6-41: Prenatal toxicity rabbit (1st study) – overview of foetal findings and abnormalities

Dose level (mg/kg bw/d)	0	25	75	150
Number of foetuses/litters examined	113/16	104/15	102/15	125/16
External findings				
Total foetuses/litters affected	2/2	1/1	0	2/2
% foetuses/litters affected	2/13	1/7	0	2/13
Omphalocele	1/1	0	0	0
Omphalocele and mandibular hypoplasia	0	1/1	0	0
Arthrogryposis of forelimbs	1/1	0	0	1/1
Right forelimbs with 1st and 5th digit missing, cleft lip (unilateral) and cleft palate	0	0	0	1/1
Visceral findings				
Total foetuses/litters affected	1/1	0	0	4/2
% foetuses/litters affected	1/6	0	0	3/13
Agenesis of kidney and ureter	1/1	0	0	0
Hypoplasia of kidneys	0	0	0	1/1
Microphthalmia (bilateral)	0	0	0	1/1
Microphthalmia (bilateral) and internal hydrocephalus	0	0	0	1/1 (2/2 ?)[$]
Skeletal findings				
Total foetuses/litters affected	4/3	2/2	3/3	3/3
% foetuses/litters affected	4/19	2/13	3/20	3/19
Irregular/asymmetrical ossification of single sternebrae	3/2	1/1	1/1	1/1
Irregular ossification of sternum	0	1/1	0	1/1
Brachymelia and sternum poorly ossified	0	0	0	1/1
Sternebrae nos. 4 + 5 partially fused/irregularly ossified	1/1	0	1/1	0
Sternebrae nos. 4 + 5 partially fused and irregulary ossification of no. 6	0	0	1/1	0

[$] according to 2nd study report amendment

Effects on weight gain in pups were seen in all studies in rats. Reduced foetal weights were reported in both developmental studies and an increase in runt foetuses were reported in the second rat developmental study (*Anon., 1985*), and statistically significant decreases in pup weight were recorded in the two multi-generation rat studies (*Anon., 1983 and Anon., 1987*) at the high dose (up to 16.5%, but mostly less than 10%) at PND 14 and 21, and in the 1987 study also at PND 4 and 7 (F2 only). Effects on survival were also recorded in various studies. In both generations of the 1987 multi-generation study, the mean number of dead pups at birth/pups that died (until day 4) was slightly but not statistically higher at 2500 ppm when compared with control. In rabbits, the number of live foetuses per litter were slightly reduced and two foetuses were dead in one study (*Anon., 1985*).

Comparison with classification criteria – development

Several findings from all four animal studies were related to developmental effects. Post implantation loss in the form of early resorptions was seen in all developmental studies at the top dose. The effects were often within historical control ranges and/or associated with maternal toxicity, but as the effects are consistently seen in all the studies, they are not disregarded as chance findings. Skeletal abnormalities were also observed. These can be interpreted as variations or delays in development which do not warrant classification on their own, however, as with the effect on post-implantation loss, the presence of the abnormality in numerous studies and species (two rat and one rabbit study) suggests a treatment-related effect and should not be ignored. Finally, and of more significance are severe malformations seen in one study in rabbits (*Anon., 1982*): there were three cases of microphthalmia, two in combination with

internal hydrocephalus. When reviewing all the available data, a pattern of developmental effects emerges and establishes a need for classification.

There was no information on the potential of penconazole to adversely affect development in humans and therefore classification in Category 1A is not applicable.

Classification in Category 1B (presumed human reproductive toxicant) is also not considered appropriate, as classification in this category should be largely based on data that provide clear evidence of an adverse effect on development in the absence of other toxic effects. Clear and consistent effects in the absence of maternal toxicity were not observed, but rather a pattern of weak effects which were considered relevant but insufficient for Category 1B.

Classification in Category 2 (suspected human reproductive toxicant) can apply where there is some evidence of an adverse effect on development, which is not sufficiently convincing to place the substance in Category 1. RAC considers the weight of evidence to be of concern and to support the classification of penconazole for developmental toxicity into Cat. 2. Therefore RAC concludes that penconazole warrants a classification as **toxic for reproduction in Category 2, H361d ("Suspected of damaging the unborn child")**; in agreement with the DS.

Assessment of effects on or via lactation

There is no human evidence to indicate a hazard to babies.

In the 1987 rat multi-generation, reduced body weight gain of pups during lactation was reported in both generations at the top dose of 2500 ppm. Also noted in both generations, the mean number of dead pups at birth/pups that died (until day 4) was slightly but not statistically higher at 2500 ppm when compared with the control group. There was no indication that treatment affected nursing behaviour of the dams.

No information was available on the quantity or quality of the milk produced by the dams, nor was the rat milk analysed for the presence of Penconazole or metabolites. Absorption, metabolism, distribution and excretion studies indicate Penconazole is extensively absorbed and distributed in body tissues and quickly eliminated while subject to extensive biotransformation. The available data is inconclusive with respect to linking the effects in the post-natal rat pup to lactation. Transfer of the active substance into milk has not been demonstrated. Therefore, RAC proposes that **no classification of Penconazole for "adverse effects on or via lactation" is warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

A harmonised classification and labelling for penconazole has been adopted by the ECHA Committee for Risk Assessment (RAC) on 11th of July 2012 (ECHA/RAC/CLH-O-0000002679-61-01/F). The substance was classified as Aquatic Acute 1, H400, M=1 based on *Lemna gibba* with 14 d EC_{50} value of 0.19 mg/L (frond numbers) and Aquatic Chronic 1, H410, M=1 based on *Daphnia magna* with 21 d NOEC value of 0.069 mg/L, not rapidly degradable and low bioaccumulation potential.

The substance is currently listed in Annex VI of Regulation (EC) No 1272/2008 with a classification for environment hazard as indicated above. Based on re-evaluation of all relevant acute and chronic aquatic toxicity studies that were provided for the renewal of the approval of the penconazole, the same classification of the substance was proposed by the RMS/DS.

The Dossier Submitter (DS) proposed to classify the substance as:

- Aquatic Acute 1, H400, M=1 based on 96 h LC_{50} value of ≤ 1.3 mg/L for *Oncorhynchus mykiss* and
- Aquatic Chronic 1, H410, M=1 based on 21 d NOEC value of ≤ 0.069 mg/L for *Daphnia magna* and no rapid degradation.

It is noted that during the process of preparation of a draft opinion, more information became available and based on this, RAC revised the DS's proposal accordingly (see Section on 'Additional key elements' below)

Degradation

Two studies carried out according to OECD TG 111 were provided for the hydrolysis of penconazole. One study showed that penconazole was stable to hydrolysis at pH 4, 5, 7, and 9 at 50 °C for 7 days and the other study at pH 5, 7 and 9 at 25 °C for 30 days. Penconazole is therefore considered as hydrolytically stable under environmentally relevant pH conditions. No hydrolysis products were detected in either of the studies.

A ready biodegradation of penconazole was determined according to OECD TG 301B (CO_2 Evolution (Modified Sturm Test)) over 29 days at 20 ±2ºC. Penconazole reached a mean of -9% ThCO_2 by day 29 (negative values are a consequence of comparison of values obtained in the blank controls and the low values obtained in presence of test item) and can be considered not readily biodegradable.

In an aerobic mineralisation in surface water study carried out in line with OECD TG 309 the mineralisation rate and route for degradation of penconazole was investigated in Fountains Abbey natural water plus suspended sediment system at two concentrations (10 and 95 µg/L) for 59 days at 20°C. The study shows that mean levels of penconazole remained similar throughout the incubation period for both concentrations and no significant degradation of penconazole was observed, therefore no characterisation of metabolites was required. Penconazole was measured to range from mean 92.7-97.0% AR (low concentration) and 93.3-95.8% AR (high concentration). Low levels of volatile radioactivity were observed, < 1.5% AR. This suggest that penconazole is stable to aerobic mineralisation in surface water. The degradation rates (DT_{50}) of penconazole were estimated using CAKE software by fitting single first-order kinetics (SFO) to the data. However, the degradation rates could not be accurately determined.

Two water/sediment simulation studies carried out according to OECD TG 308 were run for 100 days (Brands, 2009) and 706 days (Mamouni, 1998) at 20°C in the dark using two different water-sediment systems. Both studies show that penconazole dissipated rapidly from the water phase to sediment. In Mamouni (1998) the route of degradation was similar in both water/sediment systems with CGA179944 as the major degradation product observed. In one of the water/sediment systems the metabolite reached a maximum of 22.1% AR in the total system, in the other system a maximum of 5.8% AR. In Brands (2009) no metabolites were detected in either the water layer or the sediment extract of both systems. No significant mineralization was observed in both studies (8.4% (river) and 4.6% (pond) over 706 days and ≤ 0.1% in both test systems over 100 days). The kinetic analysis of both studies was performed. In Mamouni (1998) recalculated DT_{50} values were 1.88 days and 5.32 days for river and pond systems, respectively.

Total system half-lives were 563 and 1150 days for river and pond systems, respectively. In Brands (2009) recalculated DT_{50} values were 7.77 days and 17.1 days for Goover and Schoonrewordsewiel, respectively. Corresponding total system half-lives were 2010 and above 10000 days, respectively.

The DS concluded that penconazole is considered to be not rapidly degradable in the aquatic environment.

Bioaccumulation

Penconazole has a measured octanol-water partition coefficient (log K_{OW}) of 3.8 at 20°C and pH 6.9 (OECD 107, shake flask method).

A fish bioaccumulation study with bluegill sunfish (*Lepomis macrochirus*) is available for penconazole. The maximum whole fish bioaccumulation factor (BCF) of 320 was derived. The study was considered not valid and not reliable by DS as the study had deficiencies, e.g., lack of measurement of total organic carbon (TOC), growth and lipid content of fish. These are parameters which may have a direct effect on the calculated BCF and may have contributed to an underestimation of the BCF in the available study.

The DS concluded that based on a measured log K_{ow} less than 4, penconazole does not have potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Reliable aquatic toxicity data are available for penconazole technical, its metabolites and formulation A6209G for all three trophic levels (fish, invertebrates, algae and aquatic plants). A summary of the relevant information on aquatic toxicity for penconazole and formulation is provided in the following Table (the key endpoints used in hazard classification are highlighted in bold). The data for metabolites are not summarised in the Table, as based on data provided in CLH report, the DS concluded that toxicity studies with different metabolites derive effect values higher (namely, much lower toxicity) than for penconazole technical and, thus, not considered further with regard to the classification.

Table: *Summary of relevant information on aquatic toxicity of penconazole technical and formulation A6209G*

Method	Test material	Species	Endpoint	Toxicity value (mg/L)	Reference/Relevant study
Short term toxicity					
OECD TG 203 (1981)	A6209G formulation (Topas 100 EC) Purity: 100 g/L penconazole	*Oncorhynchus mykiss*	96h LC_{50}	> 5.6 - < 6.8 (f, n) > 0.56 - < 0.68 (a.s., n)	1984; CGA71818/0005 Reliable
OECD TG 203 (1981)	A6209G formulation (Topas 100 EC) Purity: 99%	*Cyprinus carpio*	96h LC_{50}	> 10 - < 12.1 (f, n) > 1.0 - < 1.21 (a.s., n)	1984a; CGA71818/0006 Reliable
OECD TG 203 (1981)	Penconazole tech. Purity: 87.3 %	*Onchorynchus mykiss*	96h LC_{50}	**≤ 1.3 (im)**	1984; CGA71818/0073 Reliable
OECD TG 203 (1981)	Penconazole tech. Purity: 99 %	*Cyprinus carpio*	96h LC_{50}	3.8 (n)	1984a; CGA71818/0076 Reliable
OECD TG 202 (1984)	A6209G formulation (Topas 100 EC) Purity: 100 g/L (n)	*Daphnia magna*	48h EC_{50}	36 (f, n) 3.88 (a.s., n)	Palmer et al, 2001; CGA71818/4379 Reliable
US EPA-660/3-75-009	Penconazole tech. Purity: unknown	*Daphnia magna*	48h EC_{50}	6.75 (n)	Hitz, 1981; CGA71818/0079 Supportive
OECD TG 201 (1984)	A6209G formulation	*Scenedesmus subspicatus*	72h E_rC_{50}	7.9 (f, n) 0.79 (a.s., n)	Memmert &Knoch, 1994; CGA71818/1234 &

Method	Test material	Species	Endpoint	Toxicity value (mg/L)	Reference/Relevant study
Short term toxicity					
	(Topas 100 EC) Purity: 100 g/L (n)		72h E_bC_{50}	3.9 (f, n)	Schuster, 2016; A6209G_11142 Reliable
OECD TG 201 (1984) OPPTS 850.5400, C.3 (1996)	Penconazole tech. Purity: unknown	*Pseudokirchneriella subcapitata*	72h E_rC_{50} 72h E_bC_{50}	4.9 (mm) 2.0 (mm)	Desjardins et al, 2001; CGA71818/4378 & Schuster, 2016; CGA071818_10472 Reliable
OECD TG 201 (2006)	Penconazole tech. Purity: 99.86%	*Pseudokirchneriella subcapitata*	72h E_rC_{50} 72h E_yC_{50}	3.41 (mm) 0.42 (mm)	Kley &Wydra, 2009: CGA071818_10633 & Lührs & Wydra, 2018: CGA071818_10633 Reliable
OECD TG 201 (2006/2011)	Penconazole tech. Purity: unknown	*Pseudokirchneriella subcapitata*	72h E_rC_{50}	3.62 (mm)	Durjava et al., 2013: ATLA, 41:65-75 Supportive
Long term toxicity					
Test was conducted to an internal protocol	Penconazole tech. Purity: 87.3%	*Pimephales promelas*	35d NOEC 35d EC_{10} 35d EC_{20}	0.36 mm 0.43 mm 0.603 mm	1984c; CGA71818/0074 & 2016; CGA071818_10494 Reliable
No specific guideline reported	Topas 100 EC Purity: NA	*Danio rerio*	96h NOEC 96h LC_{50}	< 0.8 (a.s. n) ≤ 3.73 (a.s.,n)	Aksakal &Ciltas, 2018; Chemosphere, 200:8-15 Supportive
OECD TG 234, draft ver. 2 (2010)	Penconazole tech. Purity: 97.4%	*Pimephales promelas*	98d NOEC (apical endpoints) 98d NOEC (mechanistic, VTG)	0.60 (mm) 0.28 (mm)	2012; CGA71818_10278 Reliable
Internal method US EPA-660/3-75-009	Penconazole tech. Purity: 87.3%	*Daphnia magna*	21d NOEC	**≤ 0.069 (mm)**	Surprenant, 1984d; CGA71818/0080 Reliable
OECD TG 211 (1984)	A6209G formulation (Topas 100 EC) Purity: 100 g/L penconazole (n)	*Daphnia magna*	21d NOEC 21d EC_{10}	0.32 (f, n) 0.032 (a.s., n) 0.49 (f, n) 0.049 (a.s., n)	Memmert & Knoch, 1994a; CGA71818/1235 Reliable
OECD, proposal for Toxicity Test with Chironomidae (May 1998)	Penconazole tech. Purity: 97.4%	*Chironomus riparius*	28d NOEC	0.8 (im)	Grade, 1999; CGA71818/1390 Reliable
OECD TG 201 (1984)	A6209G formulation (Topas 100 EC) Purity: 100 g/L (n)	Scenedesmus subspicatus	72h E_rC_{10} 72h E_bC_{10} 72h NOEC	3.1 (f, n) 1.6 (f, n) 1.0 (a.s., n)	Memmert & Knoch, 1994; CGA71818/12 34 & Schuster, 2016; A6209G_1114 2 Reliable
OECD TG 201 (1984) OPPTS 850.5400, C.3 (1996)	Penconazole tech. Purity: NA	Pseudokirchneriella subcapitata	72h E_rC_{10} 72h E_bC_{10} 72h NOEC	2.39 (mm) 0.50 (mm) 0.56 (mm)	Desjardins et al, 2001; CGA71818/43 78 & Schuster, 2016; CGA071818_ 10472 Reliable
OECD TG 201 (2006)	Penconazole tech. Purity: 99.86%	Pseudokirchneriella subcapitata	72h E_rC_{10} 72h E_yC_{10} 72h NOEC	0.26 (mm) 0.10 mm) 0.234 (mm)	Kley & Wydra, 2009: CGA071818_ 10633 & Lührs & Wydra, 2018: CGA071818_ 10633 Reliable

Notes: n – nominal concentration; im – initial measured concentration; mm – mean measured concentration; (f) – formulation; (a.s.) – active substance;

Acute aquatic toxicity

Two acute toxicity studies with two different fish species (*Oncorhynchus mykiss* and *Cyprinus carpio*) using penconazole technical were available. Also two toxicity studies on fish (*Oncorhynchus mykiss* and *Cyprinus carpio*) with formulation A6209G are provided. All the studies followed OECD TG 203. Rainbow trout *Oncorhynchus mykiss* was the most sensitive fish species tested in the acute studies with penconazole technical (87.3% purity), with initial measured 96 h LC_{50} value of ≤ 1.3 mg/L (95% C.I. ≤ 1.0 to ≤ 1.6 mg/L) (Anon., 1984). The study was considered reliable and valid although one validity criteria of OECD TG 203 was not fulfilled as the concentrations were not measured at the test end. Thus, is not possible to determine an accurate LC_{50}. In the study the initial measured concentration of the top dose was 108% of nominal, whereas the remaining four concentrations were between ~60-70% of nominal. Based on the whole dataset for aquatic studies conducted with penconazole technical (excluding study on *O. myikss* and studies considered not reliable) DS concluded that in the majority of the studies concentrations were not maintained. This supports the RMS view that it cannot be excluded that the true endpoint of the study with *O. mykiss* may be below 1 mg/L. In addition, two acute toxicity studies with fish using formulation A6209G resulted in nominal 96 h LC_{50} value > 5.6 and < 6.8 mg formulation/L (equivalent to > 0.56 and < 0.68 mg a.s./L) for *Oncorhynchus mykiss* and nominal 96 h LC_{50} value > 10 and < 12.1 mg formulation/L (equivalent to > 1.0 and < 1.21 mg a.s./L) for *Cyprinus carpio*. The endpoints derived from these studies also indicate that rainbow trout is a more sensitive species than carp, and that relying on the active substance study with carp for classification is not conservative. The endpoint from the study with *O. mykiss* using the formulation A6209G is below 1 mg a.s./L, when expressed in terms of the active substance. However, DS pointed out that there seem to be a general trend that the studies conducted with the formulation A6209G provides lower endpoints when expressed in terms of active substance, than the studies conducted with the active substance (technical). Hence relying on the acute toxicity study on *O. mykiss* with formulation A6209G alone for the classification of the active substance should be made with cation.

There was one study with penconazole technical (purity unknown) available for *Daphnia magna*, conducted according to US EPA-660/3-75-009, with a nominal 48 h EC_{50} value of 6.75 mg/L. The provided value is in the same range as nominal 48 h EC_{50} value of 36 mg/L for formulation A6209G (equivalent to 48 h EC_{50} value of 3.88 mg/L for active substance) from the study following OECD TG 202 with *Daphnia magna*.

Three acute toxicity studies with green algae *Pseudokirchneriella subcapitata* using penconazole technical and one study with green algae *Scenedesmus subspicatus* using formulation A6209G were available. All studies followed OECD TG 201 and one study also OPPTS 850.5400, C.3. The lowest acute endpoint for algae is mean measured 72 h E_rC_{50} of 3.41 mg/L for *Pseudokirchneriella subcapitata* using penconazole technical (purity 99.86%).

There was only one study following OECD TG 221 and US EPA Proposed Guidelines for Registering Pesticides in the United States, Subpart J, 1980; Holst RW and TC Ellwanger, 1982 available using penconazole technical (purity 87.3 %) for aquatic plants with the lowest value for acute toxicity to freshwater plant of 14 d EC_{50} value of 0.19 mg/L based on frond number for *Lemna gibba*. The study was the key study for classification of the substance for acute aquatic hazards in the previous RAC opinion for penconazole (2012). Based on re-evaluation of the study the RMS/DS concluded that the study could not be considered reliable and valid due to the following major deficiencies: the validity criteria of OECD TG 221 were not met, and exposure concentrations were not analytically verified. Therefore, the study was not taken into account for the classification of the substance.

From the available aquatic toxicity data and based on justification provided above in regard to fish studies the DS concluded that fish are the most acutely sensitive taxonomic group. The lowest acute toxicity value is 96 h LC_{50} value of ≤ 1.3 mg/L for rainbow trout *Oncorhynchus mykiss* using penconazole technical. The value is close to or possible also below the classification threshold value of 1 mg/L, therefore the classification of penconazole as Aquatic Acute 1 (H400) with M-factor 1 (0.1 < L(E)C_{50} ≤ 1 mg/L) is warranted.

Chronic aquatic toxicity

Two chronic toxicity studies with fathead minnow (*Pimephales promelas*) using penconazole technical and one study with zebrafish (*Danio rerio*) using formulation A6209G were available. The studies were conducted according to international protocol or no specific guideline or OECD TG 234. The lowest chronic endpoint for fish is mean measured 35 d EC_{10} of 0.43 mg/L for *Pimephales promelas* using penconazole technical (purity 87.3%) from the study conducted to an international protocol and evaluated according to OECD TG 210 by the DS.

Two chronic toxicity studies with two different invertebrate species (*Daphnia magna* and *Chironomus riparius*) using penconazole technical and one study on crustacea *Daphnia magna* using formulation A6209G were available. The lowest chronic endpoint for invertebrates is mean measured 21 d NOEC of ≤ 0.069 mg/L for *Daphnia magna* using penconazole technical (purity 87.3%) from the study conducted according to OECD TG 202 (Surprenant, 1984d). This result is supported by study on *Daphnia magna* with formulation A6209G that, despite associated uncertainties (presence of co-formulants), provide toxicity within the same range (nominal 21 d EC_{10} = 0.049 mg formulation/L, equivalent to: 21 d EC_{10} = 0.049 mg a.s./L and nominal 21 d NOEC = 0.32 mg formulation/L, equivalent to: 21 d NOEC = 0.032 mg a.s./L).

Two toxicity studies with green algae *Pseudokirchneriella subcapitata* using penconazole technical and one study with green algae *Scenedesmus subspicatus* using formulation A6209G were available. The lowest chronic endpoint for algae is mean measured 72 h E_rC_{10} of 0.26 mg/L for *Pseudokirchneriella subcapitata* using penconazole technical (purity 99.86%) from the study carried out according to OECD TG 201.

From the available chronic toxicity data, the DS concluded that invertebrates are the most chronically sensitive taxonomic group. The lowest chronic toxicity value is 21 d NOEC of ≤ 0.069 mg/L for *Daphnia magna* using penconazole technical. The value is supported by 21 d EC_{10} value of 0.049 mg a.s./L for *D. magna* from the study with formulation A6209G. The 21 d NOEC value of ≤ 0.069 mg/L is below the classification threshold value of < 0.1 mg/L, therefore the classification of penconazole as Aquatic Chronic 1 (H410) with M-factor 1 (0.01 < NOEC ≤ 0.1 mg/L) is warranted.

Comments received during consultation[3]

Comments were received from one Member States (MS) and one National Authority (NA). MS agreed with DS proposal for the aquatic acute classification and with the aquatic chronic classification.

NA questioned the conservative approach used by DS for the aquatic acute classification using 96 h LC_{50} ≤ 1.13 mg/L (based on initial measured concentrations and adjustment for purity) for

[3] *Note: an outcome of the statistical re-evaluation of the chronic toxicity study on Daphnia magna (Surprenant, 1984d) and a new chronic toxicity study with Daphnia magna were not available during the consultation.*

O. mykiss (Anon., 1984, report no BW-84-5-1583). NA noted that analytical verification at termination was not included and a mean measured endpoint may be below 1 mg/L. Nevertheless, for the NA it was unclear if this position is justified given i) it is unclear if test concentrations would have declined by ≥ 20% over the study, and ii) RAC previously (RAC Opinion on penconazole, ECHA/RAC/CLH-O-0000002679-61-01/F, 2012) noted that purity corrections were not required when measured concentrations were available.

NA asked for wider information to support the DS proposal that an initial measured 96 h LC_{50} of ≤ 1.3 mg/L is confidently expected to be below 1 mg/L, based on actual penconazole concentrations.

DS supported the position with the available data. For example, DS indicated that the study with the representative formulation A6209 on *O. mykiss* (reference AFT-84-056) provides results for an active substance being below 1 mg a.s./L. In addition, in four of the aquatic studies performed with technical penconazole (Surprenant D.C., 1984c., Surprenant D.C., 1984d, Grade R., 1999 and Kley A. & Wydra V., 2009) and in several studies with the representative formulation, the concentrations of technical penconazole fell below 80% at the end of the test. Thus, there will be an uncertainty regarding the stability of technical penconazole in the current study by Anon., 1984 (reference BW-84-5-1583). In addition, in acute toxicity study with *O. mykiss* all test concentrations fell below LOQ at the end of the test (Anon, 1984, report no. 840735; considered not valid by RMS).

DS explained that the agreed endpoint from the study by Anon., 1984 in the previous DAR (2008) was 1.13 mg a.s./L (initial measured) and has been corrected for purity. However, DS is of the view that purity correction is not necessary as the concentrations have been measured, thus corrected the endpoint to ≤ 1.3 mg a.s./L (initial measured).

NA noted that the study by Anon., 1984 appears to have been static, but the CLH report describes it as semi-static. NA asked for clarification regarding test design as this impacts the potential loss of test substance. DS clarified that the test design is static.

In addition, NA indicated that the RAR description includes initial measured 96 h LC_{50} of 1.2 mg/L (95% C.I. 1.0-1.6) in Table 9.2.1-3. NA asked for clarification whether the 96 h LC_{50} is 1.2 mg/L or 1.3 mg/L (im) and associated confidence intervals. DS clarified that the value of 1.2 mg/L is a typo.

NA also highlighted that the study by Anon., 1984 employed penconazole at 87.3% purity (below the specification purity in the CLH report) and asked whether there is information to consider impurity toxicity? DS responded that an assessment of whether the impurities in the batch FL 830634 may have contributed to the toxicity observed in the study has not been done.

NA pointed a further non-GLP acute toxicity to fish (*Cyprinus carpio*) study (Anon, 1984a, report no 840736 in the CLH report) using penconazole (99% purity) resulted in nominal 96 h LC_{50} of 3.8 mg/L (95% C.I. 2.5-5.2 mg/L). In this study OECD TG 203 validity criteria were met, and the study included analytical verification at termination. With the exception of the lowest treatment (76% nominal), measured concentrations were within 20% of nominal. Noting this, it would appear that an LC_{50} based on mean measured concentrations would be more appropriate for hazard classification (ECHA, 2017) but this is unlikely to result in a LC_{50} < 1 mg/L, according to the NA. DS agreed that this is a valid fish study with an endpoint clearly above 1 mg a.s./L, however DS noted that there is evidence from studies with active substance and formulation that *O. mykiss* is more sensitive. Thus, basing the classification of the penconazole on the study with *C. carpio* is not sufficiently protective.

NA pointed also that a further non-GLP static acute toxicity to O. mykiss study (Anon, 1984; report no 840735 in the RAR) is available using penconazole at a higher purity (99%) and appears to be relevant for hazard classification. The quoted study 96 h LC_{50} was 4.3 mg/L with a NOEC of 3.2 mg/L (based on nominal concentrations). In this study OECD TG 203 criteria were met although > 20% loss to < 0.5 mg/L was observed over the study duration for all treatments (1.0-10 mg/L nominal). NA proposed to calculate the LC_{50} based on measured concentrations using half of the detection limit for LOD following ECHA guidance, 2017. DS pointed out that the study was not considered valid as concentrations fell below the detection limit at the end of the test. DS indicated that as only two measurements were performed (at the beginning and at the end of the test), it could not be known if the decline of the test substance occurred short after the start of the study or towards the end. Thus, such an endpoint will be highly uncertain. EFSA requested the applicant to provide a new acute toxicity study with technical penconazole and *O. mykiss* in order to fulfill the data requirement of Commission Regulation 283/2013 or in case the study is not available to provide the clear and valid justification on why the acute study with Carp and the technical penconazole is suitable as a substitute for the study with Rainbow trout.

NA indicated that based on these three studies (Anon, 1984, report no BW-84-5-1583; Anon, 1984a, report no 840736 and Anon, 1984, report no 840735), and given penconazole appeared stable in wider acute ecotoxicity testing (e.g. OECD TG 201 and 202) it is unclear if the penconazole acute toxicity to fish endpoint should conservatively be considered below 1 mg/L.

NA questioned whether the mean measured 21 d NOEC of ≤ 0.069 mg/L for *Daphnia magna* from the chronic toxicity study (Suprenant, 1984) is a true NOEC as it was the lowest treatment, or whether the true NOEC would be lower. NA asked for confirmation if the repeat study that is mentioned in the CLH report (due for complete in 2022) is now available along with any additional recent ecotoxicity information. DS provided the information that applicant has carried out chronic toxicity study with *Daphnia magna* using technical penconazole and according to OECD TG 211 and GLP compliant and that the study could be submitted by applicant on request by EFSA. EFSA requested the applicant to provide a new chronic study and statistical re-evaluation of the study by Suprenant (1984d) during the Stop-the-clock (1st quarter of 2023).

NA asked for any information on co-formulants in the formulation used in the chronic toxicity study on *Daphnia magna* (Memmert & Knoch, 1994; used as supporting study) to consider their potential impact on the study endpoint (21 d NOEC of 0.032 mg a.s./L / EC_{10} of 0.049 mg a.s./L). DS responded that co-formulants are given in Table C.1.5.1.2-1 of Vol. 4 for the applicant in the RAR. One co-formulant is classified with H412, the remaining co-formulants have no classification with regard to the aquatic environment.

Additional key elements

During the process of preparation of a draft opinion, RAC became aware of additional information generated during the procedure for renewal of the approval of penconazole in accordance with Commission implementing regulation (EU) No 844/2012. This additional information was submitted in April 2023 and was reviewed by RAC. Additional information provided refers to four new experimental studies performed with penconazole and statistical re-calculation of the OECD 211 study by Suprenant (1984d). A brief description of documents/studies is provided in "Supplementary information" in the Appendix.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider penconazole as not rapidly degradable. The substance is hydrolytically stable at environmentally relevant pHs (pH 5-9) and is not readily biodegradable. No significant degradation in the aerobic mineralisation study was observed. The results of water/sediment simulation study show that penconazole dissipates rapidly from the water phase to the sediment phase. Mineralisation of penconazole was low. Total system DT_{50} values were in the range of 563 to > 10000 days.

Bioaccumulation

RAC agrees with DS that BCF study with *Lepomis macrochirus* may be considered as not valid. RAC agrees also that penconazole does not fulfil the criteria for bioaccumulating potential based on log K_{ow} value of 3.8 which is below the CLP Regulation criterion of 4.

Aquatic toxicity

During the preparation of the draft opinion the new toxicity studies with penconazole on algae *Desmodesmus subspicatus*, aquatic plant *Lemna gibba* and invertebrate *Daphnia magna* were provided by the PPP Applicant to EFSA/ECHA (see additional key elements and Appendix). In addition, statistical re-analysis of the existing chronic toxicity to *D. magna* (Suprenant, 1984) was also provided. RAC evaluated the additional studies and data for penconazole and considers them adequate. Therefore, it is appropriate to consider them relevant for classification of the substance.

RAC notes that the EC_{50} value from new acute toxicity study with *Lemna gibba* (7d E_rC_{50} = 0.755 mg/L based on frond number) and EC_{10} value from the new chronic daphnia study (21 d EC_{10} = 0.010 mg/L based on length) are lower that the key values used for the proposal for classification of the substance for acute and chronic hazard by the DS (96 h $LC_{50} \leq 1.3$ mg/L for *Oncorhynchus mykiss* and 21 d NOEC ≤ 0.069 mg/L for *Daphnia magna*).

In consequence, both studies will be used as the basis for aquatic hazard classification. Although, the new study on *Lemna gibba* does not change the acute classification of penconazole as proposed by the DS, but the new chronic daphnia study leads to a higher M factor for the chronic classification of penconazole.

RAC is of the opinion that reliable acute and chronic toxicity data on penconazole are available for fish, invertebrates, algae and aquatic plants.

Acute toxicity

Aquatic plants are the most sensitive taxonomic group, and the lowest result is a geometric mean measured 7d E_rC_{50} value of 0.755 mg/L (frond number) from new study with penconazole on duck weed *Lemna gibba*. Based on 7d E_rC_{50} value of 0.755 mg/L, which is below the $EC_{50} \leq 1$ mg/L criterion, RAC concludes that penconazole **warrants classification as Aquatic Acute 1 (H400) with M-factor of 1 (0.1 < L(E)C50 ⩽ 1 mg/L) for acute aquatic hazard.**

Chronic toxicity

Penconazole is considered not to be rapidly degradable and does not fulfil the criteria for bioaccumulating potential. Based on all available data, RAC proposes to base the aquatic chronic classification of the susbtance on the new chronic toxicity study with penconazole performed on

Daphnia magna with a nominal 21 d EC_{10} value of 0.010 mg/L based on length. Based on 21 d EC_{10} value of 0.010 mg/L, which is below the NOEC or ECx of ≤ 0.1 mg/L criterion, RAC concludes that penconazole **warrants classification as Aquatic Chronic 1 (H410) with M-factor of 10 (0.001 < NOEC ⩽ 0.01).**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

No classification for hazards to the ozone layer was proposed by the DS.

The degradation of the penconazole in the atmosphere was determined with Atmospheric Oxidation Program (AOP, version 1.85 and 1.91), based on the Atkinson model. The half-life (DT50) of penconazole in the atmosphere was calculated to be 1.32 days at an assumed average atmospheric OH concentration of 1.5×10^6 cm^{-3} for a 12-hour day, and 1.99 days assuming an average atmospheric concentration of 0.5×10^6 OH radicals cm^{-3} for a 24-hour day. The dominant degradation process for penconazole in the atmosphere is considered to be via reaction with OH (via alkyl hydrogen abstraction and aromatic–ring-addition mechanisms). Penconazole has a low vapour pressure of 9.4×10^{-5} Pa at 20°C. The Ozone Depleting Potential (ODP) is not reported for penconazole.

It should also be noted that penconazole is not listed in Appendix I of Regulation No 1005/2009 of the European Parliament and of the council (16 September 2009).

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Penconazole is not expected to remain stable in the air based on half-life of 1.99 days. Due to its low half-life in the atmosphere combined with a low vapour pressure (9.4×10^{-5} Pa at 20°C) indicating non-volatility and resulting in a low value for the Henry's Law constant (3.4×10^{-4} Pa m^3/mol at 20°C), penconazole is considered not to be subject to transport via air or cause hazard to ozone layer.

Therefore, RAC agrees with the DS view that penconazole does not fulfil the criteria for classification as 'hazardous to the ozone layer'.

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 and additional information (when applicable).

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

Appendix

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

Additional key elements

Summary of studies presented from the RAC opinion (2012)

The dog appeared to be the most sensitive species. In a study conducted according to a protocol similar to OECD Guideline No. 409 (Anon., 1984), the derived 90-day NOAEL for males and females were 3.1 and 3.3 mg/kg bw/day (100 ppm), respectively. The associated LOAELs of 16.9 and 16.7 mg/kg/day were based on hepatotoxicity effects: Inflammatory cell infiltration, necrosis, clear dose-dependent increase in liver weight. Also, an increase in the activities of alkaline phosphatases γ-GT, AST, and ALT was observed. However, most of these signs were not severe. Furthermore, when incidence is estimated by pooling males and females, the single incidence of necrosis appears to be an isolated case: 1/8 after 90 days, 0/8 after 1 year.

In this study it was also observed in high dose males' group a moderate to marked reduction in spermatogenic activity, characterised by atrophy of the seminiferous epithelium associated with formation of giant cells, and absence of spermatozoa in the epididymis (which contained cellular debris). However, the 5000-ppm dose is largely above the MTD that was estimated around 2500 ppm. In the lower/intermediate/high dose groups, some decreases in relative gonad weights were also observed, but the observations were inconsistent compared to control: +23%, -4% and -27% for males and -35%, -8%, -16% in females at the 90-day time point. On the other hand, the liver weight increase was clearly dose-dependent: +1, +15, +75% for males and +8, +24, +88% for females.

In a 90-day oral rat study, conducted according to OECD Guideline No. 408 and with Penconazole with a purity of 98.7% (Anon., 1987a), evidence of hepatotoxicity was also found. Observations include dose-related centrilobular hypertrophy of hepatocytes (in males 0/15, 3/15, 12/15 and 15/15 for 300, 500, 1000 and 2400 ppm, weaker in females), hepatocellular degeneration around the central vein, and an increase in the incidence of hepatocytic vacuolisation (in males 0/15, 1/15, 5/15 for 500, 1000 and 2400 ppm, weaker in females). The derived NOAELs for male and female rats were 23.2 and 28.3mg/kg bw/day (300 ppm), respectively. The LOAELs were 37.5 and 45.2 mg/kg bw/day (500 ppm). A very similar picture was also observed in mouse liver: dose dependent increase in absolute and relative liver weight (statistically significant from 500 pmm in males and 2400 in females), centrilobular hypertrophy of hepatocytes (in males 0/15, 3/15, 6/15 and 14/15 for 300, 500, 1000 and 2400 ppm), and hypertrophic hepatocytes around the central vein with some vacuolar (2400 ppm, males only) (Anon.,1987b, according to guideline similar to OECD guideline No. 408).A summary table of the all studies in this category can be seen below.

***Table**: Summary of studies applicable to STOT RE*

Method, guideline,	**Route of exposure, Duration**	**Species, Strain, Sex, No/group**	**Dose Levels ppm (mg/kg bw/day)**	**NOAEL ppm (mg/kg bw/day)**	**LOAEL ppm (mg/kg bw/day)**	**Results Main effects/Target organs**	**Reference**
Oral Cumulative Toxicity Study In Rats OECD 407:	28-Day, Oral (gavage),	Tif:RAIf (SPF) 10M+10F	First week 0, 20, 100, 500 From 2nd week: 0, 100, 500, 1000	20 < 100	100 < 500	Target organ: Liver Bw gain↓, water consumption (F) ↑ *clinical parameters*:* bilirubin (F) ↓, Total protein (F) ↑, and globulin (F) ↑; *liver:* weight* ↑, hepatocellular hypertrophy; *kidney:* weight*↑, urine volume (F)↑; *adrenal*: weight* (F) ↑;	Anon., (1984) K-CA 5.3.1/01 Report No. 820822
Subacute, Oral Toxicity Study In Rats OECD 407:	Oral (gavage), 28-Day	Rat Tif:RAIf 10M+10F	0-100-500	<100	100	Target organ: Liver *Haematology parameters:* platelets ↑; prothrombin time ↓; *clinical chemistry parameters*:* total protein ↑, cholesterol ↑ *liver:* weight* ↑, hepatocellular hypertrophy; *thyroid:* weight*↑ minimal hypertrophy of the follicle epithelium (M)	Anon., (1991) K-CA 5.3.1/02 Report No. 901026
Toxicity Study In Rats Guideline not reported, OECD 408	Oral (diet) 3-Month	Rat, Tif:RAIf; 20M+20F	0-30-300-3000 ppm (M: 0-2.0-19.4-202 F: 0-2.1-20.7-209)	300 (M: 19.4; F: 20.7)	3000 (M: 202; F: 209)	Target organ: Liver Bw gain (F) ↓; *liver:* weight* ↑, hepatocellular hypertrophy; *trends in clinical chemistry parameters towards:* total protein ↑, glucose ↓, urea nitrogen↑, cholesterol ↑, albumin ↑	Anon., (1982) K-CA 5.3.2/01 Report No. 801194
Toxicity Study In Rats OECD 408	Oral (diet) 3-Month	Rat, Tif:RAIf; 20M+20F	0-10-30-100 (M: 0-0.8-2.1-7.1 F: 0-0.8-2.1-7.3)		> 100 (M: 7.1; F: 7.3)	Protein ↑	Anon., (1983) K-CA 5.3.2/02 Report No. 821054
Subchronic Toxicity Study In Albino Rats OECD 408	Oral (diet) 90-Day	Rat, Crl:CD(SD)BR 15M+15F	0-10-100-300-500-1000-2400 (M: 0-0.8-	300 (M: 23.2; F: 28.3)	500 (M: 37.5, F: 45.2)	Target organ: Liver hepatocellular vacuolisation (M)	Anon., (1987b) K-CA 5.3.2/03

Method, guideline,	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose Levels ppm (mg/kg bw/day)	NOAEL ppm (mg/kg bw/day)	LOAEL ppm (mg/kg bw/day)	Results Main effects/Target organs	Reference
			7.5-23.2-37.5-72-179 F: 0-1.0-9.8-28.3-45.2-86-209			hepatocellular hypertrophy (M, F)	Report No. HLA 6117-120
Toxicity Study In Dogs OECD 409	Oral (diet) 90 days	Dog, Beagle 4M+4F	0-100-500-5000/2500 (M**: 0-3.4-18.2-132 F**: 0-3.8-19.4-137)	100 (M: 3.3; F: 3.8)	500 (M: 17.5; F: 18)	Target organ: Liver Bw gain ↓; liver: weight↑, hepatocyte necrosis	Anon., (1984); K-CA 5.3.2/04 Report No. 801187
Toxicity Study In Dogs Not reported; OECD 409 (1981)	Oral (diet) 1 year	Dog, Beagle 4M+4F and 2M+4F for recovery	0-100-500-5000/2500 (M**: 0-3.0-16.8-108 F**: 0-3.2-16.5-110)	100 (M: 3.1; F: 3.3)	500 (M: 16.9; F: 16.7)	Target organ: Liver Bw gain ↓; liver: weight↑, hepatocyte necrosis, inflammation, fibrosis	Anon., (1984); K-CA 5.3.2/04 Report No. 801187
90-Day Subchronic Dietary Toxicity And Kinetic Study In Albino Mice OECD 408	Oral (diet) 90 days	Mouse, Crl:CD-1(ICR)BR 15M+15F	0-10-100-300-500-1000-2400 (M: 0-1.7-17.1-51.8-84.7-163-423 F: 0-2.5-23.9-72.2-115.6-237-614)	M: (500) 85; F: (1000) 237	M: (1000) 163; F: (2400) 614	Target organ: Liver *clinical chemistry parameters:* total protein ↓, albumin (F)↓ cholesterol ↓ A/G ratio (F) ↓ γ-GT (M) ↓ *liver:* weight↑, hepatocellular hypertrophy, coagulative necrosis	Anon., (1987) K-CA 5.3.2/05 Report No. HLA 6117-121
Preliminary Carcinogenicity Study In Mice OECD 408	Oral (diet) 90 days	Mouse, C57BL/10J fCD-1 10M+10F	0-100-500-1500-3000-5000 (M: 0-14-69-229-437-837 F: 0-18-87-274-545-983)	500 (M: 69; F: 87)	1500 (M: 229; F: 274)	Target organ: Liver Bw gain ↓; liver: weight↑, hepatocellular hypertrophy	Anon., (2002) K-CA 5.3.2/06 Report No. CTL/PM1235
21-Day Repeated Dose Dermal Toxicity Study in Rabbits OECD 410	Dermal 21 days	Rabbit, NZW; 5M+5F	M/F: 0-1000-1500-2000	M/F: 2000	> 2000		Anon., (1983) K-CA 5.3.3/01 Report no 820206.

RAC evaluation of aquatic hazards (acute and chronic)

Supplemental information

Determination of acute toxicity to the green alga *Desmodesmus subspicatus* (VV-880194_D subspicatus)

Test was carried out in accordance with OECD TG 201 and was GLP compliant. Green algae *Desmodesmus subspicatus* was exposed to penconazole (purity 98.5%) for 96 hours under static exposure conditions to the nominal concentrations of 1.0, 2.6, 6.4, 16, 40 and 100 mg/L and culture medium control. The measured concentrations of penconazole were maintained within ± 20% of nominal, thus the endpoints are based on nominal concentrations. The study met all validity criteria of the OECD TG 201 for 72 hours and 96 hours test duration. The results of the study are presented in the table below.

***Table**: Summary of toxicity endpoints based on nominal concentrations*

Endpoint	72 hours toxicity value (mg/L)	96 hours toxicity value (mg/L)
E_rC_{50}	> 100	> 100
E_rC_{10}	> 100	> 100
NOE_rC	100	100
E_yC_{50}	> 100	> 100
E_yC_{10}	> 100	> 100
NOE_yC	100	100
E_bC_{50}	> 100	> 100
E_bC_{10}	> 100	> 100
NOE_bC	> 100	> 100

Determination of acute toxicity to Lemna gibba (VV-912328_Lemna gibba)

Test was carried out in accordance with EPA Ecological Effects Test Guideline OCSPP 850.4400: Aquatic Plant Toxicity Test using Lemna spp. (2012) and was GLP compliant. Duckweed *Lemna gibba* was exposed to penconazole (purity 97.4%) for 7 days under semi-static (daily renewals) exposure conditions to the nominal concentrations of 0.032, 0.1, 0.32, 1.0, 3.2 and 10 mg/L and dilution water control. The measured concentrations of penconazole in the fresh 'on' test solutions during the 0-7 day exposure period ranged from 85% to 140% of nominal. The measured concentrations of penconazole in the 'off' solutions during the 0-7 day exposure period ranged from 83-122% of nominal. Based on analytical results, geometric mean measured concentrations (0.0372, 0.105, 0.304, 1.01, 2.91 and 9.92 mg/L) were used to derive the endpoint. The test met validity criteria of the EPA Ecological Effects Test Guideline OCSPP 850.4400. The results of the study are presented in the table below.

***Table**: Summary of toxicity endpoints based on geometric mean measured concentrations*

Endpoint	Toxicity value (mg/L)
Growth rate	
7 d E_rC_{50} (Frond number)	0.755 (95% CI 0.645-0.868)
7 d E_rC_{10} (Frond number)	0.0898 (95% CI 0.0655-0.106)
7 d NOE_rC (Frond number)	0.0372
7 d E_rC_{50} (Dry weight)	0.267 (95% CI 0.189-0.369)
7 d E_rC_{10} (Dry weight)	0.0171 (95% CI 0.00162-0.0427)
7 d NOE_rC (Dry weight)	Not determined
Yield	
7 d E_yC_{50} (Frond number)	0.141 (95% CI 0.124-0.160)
7 d E_yC_{10} (Frond number)	0.0303 (95% CI 0.0185-0.0417)
7 d NOE_yC (Frond number)	0.0372
7 d EyC_{50} (Dry weight)	0.102 (95% CI 0.0842-0.123)
7 d EyC_{10} (Dry weight)	0.0108 (95% CI 0.00337-0.0199)
7 d NOE_yC (Dry weight)	Not determined

Note: 95% CI - 95% confidence intervals

Determination of acute toxicity to *Daphnia magna* (VV-971642_48h D magna)

Test was carried out in accordance with OECD TG 202 and was GLP compliant. Freshwater crustacean *Daphnia magna* was exposed to penconazole (purity 97.4%) for 48 hours under static exposure conditions to the nominal concentrations of 0.512, 1.13, 2.48, 5.45, 12 and 26.4 mg/L and dilution water control. Measured concentrations were not maintained within ± 20% of nominal, and geometric mean measured concentrations (0.476, 1.03, 2.40, 5.07, 9.58 and 26.6 mg/L (sum of measured S- and R-enantiomers)) were used to derive the endpoint. The test met all the validity criteria of the OECD TG 202. The minor deviation from the guideline was reported but was deemed to have no effect on the outcome of the test. In the study, the temperature remained within 18 to 22°C but varied by a maximum of 1.1°C over the exposure period which is not in line with guideline (18 – 22°C kept constant to ± 1°C). Based on geometric mean measured concentrations of penconazole, the 48 hours EC_{50} was 6.93 mg/L (95% confidence limits 6.02 – 7.47 mg/L).

Determination of effects on reproduction to *Daphnia magna* (VV-955214)

Test was carried out in accordance with OECD TG 211 and was GLP compliant. Freshwater crustacean *Daphnia magna* was exposed to penconazole (purity 97.4%) for 21 days under semi-static (renewal 3 times a week) exposure conditions to the nominal concentrations of 0.005, 0.016, 0.050, 0.158 and 0.500 mg/L and culture medium control. The geometric mean measured values were all within ± 20% of nominal, therefore the nominal concentrations were used to

derive the endpoint. The test met all the validity criteria of the OECD TG 211. The results of the study are presented in the table below.

***Table**: Summary of toxicity endpoints based on nominal concentrations*

Endpoint	Toxicity value (mg/L)
21 d NOEC (survival)	≥ 0.500
21 d EC_{10} (total number of offspring per surviving *D. magna* at test end)	0.016 (95% CI 0.013 – 0.020)
21 d NOEC (total number of offspring per surviving *D. magna* at test end)	0.016
21 d EC_{10} (length)	0.010 (95% CI 0.008 – 0.013)
21 d NOEC (length)	0.016

Note: 95% CI - 95% confidence intervals

Penconazole – Method review and statistical re-analysis – The chronic toxicity of CGA 71818 to Daphnia magna (VV-946381)

The review and re-analysis of the available data from the original study (Surprenant, 1984) revealed that reliable values could be determined for all endpoints and are presented in the Table below.

***Table**: Toxicity values based on mean measured concentrations*

Endpoint	Toxicity value (mg/L)
21 d EC_{10} (cumulative offspring per surviving parent)	0.083 (95% CI 0.050 – 0.117)
21 d NOEC (cumulative offspring per surviving parent)	0.069
21 d NOEC (mortality/immobility)	1.6

Note: 95% CI - 95% confidence intervals