Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Prothioconazole (ISO); 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione

EC Number: -
CAS Number: 178928-70-6

CLH-O-0000001412-86-269/F

Adopted

15 March 2019

15 March 2019

CLH-O-0000001412-86-269/F

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

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

Chemical name: **Prothioconazole (ISO); 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione**

EC Number: **-**

CAS Number: **178928-70-6**

The proposal was submitted by **the United Kingdom** and received by RAC on **22 March 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 April 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 June 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Daniel Borg**

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

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2019** by **consensus**.

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

	Index No	International Chemical Identification	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	No current Annex VI entry										
Dossier submitters proposal	TBD	prothioconazole (ISO); 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione	-	178928-70-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=10 M=1	
RAC opinion	TBD	prothioconazole (ISO); 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione	-	178928-70-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=10 M=1	
Resulting Annex VI entry if agreed by COM	TBD	prothioconazole (ISO); 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione	-	178928-70-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=10 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Prothioconazole is an active substance in plant protection products used in foliar and seed treatment to control diseases caused by pathogen fungi from the classes Ascomycetes, Deuteromycetes and Basidiomycetes. It belongs to the triazolinethione class of fungicides that act by blocking sterol biosynthesis via cytochrome P450 51 (CYP51). The substance is not currently listed in Annex VI of the CLP Regulation (EC) 1272/2008.

The substance is manufactured as a 50:50 racemate of ≥ 97.0% purity containing toluene (≤ 0.5%) as the major impurity. It has moderate water solubility (22.5 mg/L, log K_{OW} 2.0 at pH 7) and low vapour pressure (1.8 x 10^{-9} Pa at 25 °C). The substance is a solid at room temperature.

Following oral administration in rats, prothioconazole shows rapid and nearly complete absorption, broad tissue distribution (primarily to liver and kidney) and almost complete excretion within 48 hours. Prothioconazole is extensively metabolised to 18 metabolites that are found, together with the parent compound, in rat urine, faeces and bile. The most abundant metabolite is prothioconazole-S-glucuronide (~46% in bile and up to 8% in the urine) followed by the unchanged parent compound (1-22%, depending on administered dose) and prothioconazole-desthio (0.4-18%). Prothioconazole-desthio was found almost exclusively in the faeces and systemically only to a very low extent (urine: max. 0.07%, bile: max. 0.45%). The S-glucuronide conjugate has a higher polarity than the parent compound and is therefore rapidly excreted. The conjugation results in the sulphur being protected against cleavage, thereby preventing modification of the triazolinethione moiety to a triazole, leading to very low amounts of prothioconazole-desthio formed in animals. Studies in liver microsomes and hepatocytes from rats and humans showed that the metabolic patterns were qualitatively very similar, with no human-specific metabolite detected, and that the principal metabolic reactions were identical in both species with S-conjugation with glucuronic acid being the major detoxification route in both species.

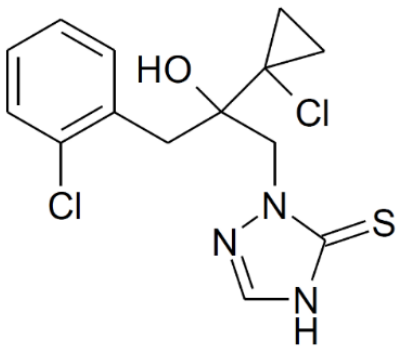

Figure 1. Molecular structure of prothioconazole.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification for physical hazards based on the following results:

- Explosive properties: negative results in EC A.14/OECD TG 113 test, Koenen test, Time pressure test, Trautzl test (*data conclusive, not sufficient for classification)*,
- Flammable solids: negative results in an EC A.10 test (*data conclusive, not sufficient for classification*)
- Self-reactive substances: negative results in two separate EC A.16 tests (*data conclusive, not sufficient for classification*)
- Pyrophoric solids: negative (*testing waived, experience from manufacturing and handling of prothioconazole have shown that the substance does not ignite in contact with air*),
- Self-heating substances: negative results in UN N.4 (Bowes-Cameron-Cage) test, EC A.16 test and EC A.1/OECD TG 102 test (*data conclusive, not sufficient for classification*)
- Substances which in contact with water emit flammable gases: negative (*testing waived, experience from manufacturing and handling of prothioconazole have shown that the substance does not react with water*),
- Oxidizing solids: negative results in an EC A.17 test (*data conclusive, not sufficient for classification*)
- Corrosive to metals: negative (*testing waived, experience from manufacturing and handling of prothioconazole have shown that the substance does not react with metallic containers*).

Due to the solid form and chemical structure of the substance, physical hazards related to gases, liquids and organic peroxides were not considered relevant for the evaluation by the DS.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

The CLP criteria for physical hazards have not been met for prothioconazole. RAC therefore agrees with the DS that **no classification for physical hazards is warranted**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

For acute oral toxicity, the DS presented one OECD TG 423 study (GLP) where 3 male and 3 female Wistar rats were given a single oral dose of 5000 mg/kg bw (actual test concentration 6200 mg/kg bw) of prothioconazole (99.8% purity). There were no deaths or clinical signs of toxicity aside from diarrhoea and a decrease in motility, which occurred in all animals in the first 1-6 hours post-dose only. Body weight gains remained normal throughout the study and no

treatment-related findings during gross necropsy were noted. Therefore, the DS concluded that the LD_{50} value for acute oral toxicity was > 6200 mg/kg bw, warranting no classification for acute oral toxicity.

For acute dermal toxicity, the DS presented one OECD TG 402 study (GLP) where 5 male and 5 female Wistar rats received a single dermal application of 2000 mg/kg bw of prothioconazole for 24 hours under a semi-occlusive dressing. There were no deaths or clinical signs of toxicity. Body weight gain during the observation period was minimal in males and absent in females. The applicant attributed this to the age of the females at dosing (15 weeks) where minimal body weight gain would be expected. Therefore, the DS concluded that the LD_{50} value for the acute dermal toxicity of prothioconazole in the rat was > 2000 mg/kg bw, warranting no classification for acute dermal toxicity.

For acute inhalation toxicity, the DS presented one OECD TG 403 study (GLP) where 5 male and 5 female Wistar rats were exposed to 5 mg/L of prothioconazole for 4 hours (nose-only) with a mass median aerodynamic diameter (MMAD) of 3.85 ± 2.06 µm. There were no deaths. Clinical signs of toxicity included pilo-erection, nasal discharge, laboured breathing, bradypnea and reduced mobility. There was also a reduction in body-weight gain and decreased body temperature. All clinical signs were resolved by day 3 and were considered non-specific responses to dust exposure. There were no treatment-related gross necropsy findings in any animal. The DS concluded that the 4-hour LC_{50} value of prothioconazole was > 5 mg/L, warranting no classification for acute inhalation toxicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The oral LD_{50} value of > 6200 mg/kg bw in a reliable study is above the cut-off criteria of 2000 mg/kg bw for classification according to CLP. RAC therefore agrees with the DS that no classification is warranted for the substance for acute toxicity via the oral route.

The dermal LD_{50} value of > 2000 mg/kg bw is in a reliable study above the cut-off criteria of 2000 mg/kg bw for classification according to CLP. RAC therefore agrees with the DS that no classification is warranted for the substance for acute toxicity via the dermal route.

For acute inhalation toxicity in a reliable study, 5 mg/L resulted in no mortality and the clinical signs of toxicity were resolved by day 3. The mean MMAD of 3.85 ± 2.06 µm was at the upper limit but within the range of 1-4 µm with a recommended standard deviation of 1.5 – 3.0 according to the OECD TG. Therefore, RAC agrees with the LC_{50} of > 5 mg/L and that no classification is warranted for acute toxicity via the inhalation route.

Overall, RAC agrees with the DS that **no classification is warranted for acute toxicity via any route of exposures**.

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

Summary of the Dossier Submitter's proposal

For the evaluation of specific target organ toxicity of prothioconazole following single exposure, the DS presented the results of four studies - the acute toxicity studies following oral, dermal and inhalation exposure as well as an acute neurotoxicity study. In the acute toxicity studies, only transient, non-adverse effects were observed.

In the acute neurotoxicity study (OECD TG 424, GLP) no deaths or effects on body weights were observed up to 2000 mg/kg bw. The only functional observation battery (FOB) signs noted were perianal stains and reduced motor and locomotor activity at 4h post-treatment only in both sexes at 750 and 2000 mg/kg bw. There were no FOB effects at 7 or 14 days. There were no gross necropsy findings, effects on brain weight, neurohistopathological changes in nerve tissue or persistent signs of neurobehavioral toxicity.

The DS did not propose any classification for STOT SE 1 or 2 based on the lack of severe target organ toxicity in the evaluated studies. For STOT SE 3, regarding narcotic effects, transiently decreased motor and locomotor activities occurred 4h post-dosing in the acute neurotoxicity at the mid and high dose study but was considered by the DS to be due to the animals feeling unwell after dosing and not a narcotic effect. For STOT SE 3, with regard to respiratory tract irritation, the observed laboured breathing, serous nasal discharge and red encrustation around the muzzle/nostrils that were observed in the acute inhalation toxicity study could indicate respiratory tract irritation. However, the effects were all reversible within three days and at necropsy, no adverse histopathological findings were observed. Thus, the DS did not propose STOT SE 3 classification.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The results of four reliable acute toxicity studies were presented. No adverse organ effects were noted, a transient decrease in motor activity was observed in the acute oral and inhalation study. In the acute inhalation toxicity study, transient irritating effects were observed, likely due to the inhalation of dust particles. A transiently decreased motor activity also was observed following oral dosing in the acute neurotoxicity study, however likely an effect of the dosing. RAC agrees with the DS that **no classification for STOT SE is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of prothioconazole (99.8% purity) was investigated in 3 male Himalayan rabbits in an OECD TG 404 study (GLP). 500 mg powder was moistened with water and applied for 4 hours under a semi-occlusive dressing. Observations for skin reactions were carried out at 1, 24, 48 and 72 hours. There were no skin reactions observed at any time point (mean score 0 in 3/3 animals). The DS thus proposed no classification for skin corrosion/irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No irritation was observed in any animal at any time point (mean irritation score 0) in a reliable study designed to test skin corrosion/irritation. The substance therefore does not meet the CLP criteria for classification.

RAC notes that in the acute dermal toxicity study, where male and female Wistar rats were exposed to 2000 mg/kg bw of prothioconazole in water for 24h under a semi-occlusive dressing, the treated skin showed signs of partial reddening (males and females) and partial scale formation (females) on days 2-8. This could indicate skin-irritating properties of prothioconazole. However, it could also be a result of the extended exposure period with the semi-occlusive dressing (24h as opposed to 4h in the OECD TG for skin irritation). In addition, there were no control animals for comparison. RAC therefore considers the results from this test insufficient for conclusions on skin irritation.

RAC agree with the DS on **no classification of prothioconazole for skin corrosion/irritation**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of prothioconazole (99.8% purity) was investigated in 3 male Himalayan rabbits in an OECD TG 405 study (GLP). 100 mg of powder was installed in one eye of each rabbit and the eyes were examined for irritation at 1, 24, 48 and 72 hours. There were no observations of corneal opacity, iritis or chemosis. One animal showed signs of minimal (grade 1) conjunctival redness at the 1-hour observation only. The DS proposed no classification for serious eye damage/irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

For classification as an eye irritant (category 2), a positive response in at least 2 out of 3 animals of corneal opacity ≥ 1; and/or iritis ≥ 1; and/or conjunctival redness ≥ 2; and/or conjunctival oedema (chemosis) ≥ 2; calculated as mean scores after grading at 24, 48 and 72 hours is required, and fully reversed within 21 days. These criteria were not met in a reliable study. RAC therefore agrees with the DS proposal that **no classification is warranted for prothioconazole for serious eye damage/irritation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitising potential of prothioconazole was investigated in a guinea-pig maximisation test (GPMT) and a modified mouse local lymph node assay (LLNA).

In the GPMT performed in accordance with OECD TG 406 (GLP), an intra-dermal induction of 5% prothioconazole (purity 99.8%) together with Freund's complete adjuvant (FCA) was administered to 10 male guinea pigs followed by a topical induction of 25% and a challenge exposure of 12%. The dose setting was based on the results of a range-finding study where skin irritation was observed at several test concentrations. In the main study, minimal skin irritation was scored in 1/10 animals 24 and 48 hours after the challenge. No irritation was recorded in the controls. Based on the response of 1/10 animals, the DS considered prothioconazole not to be a skin sensitiser under the conditions of this study.

The mouse LLNA was performed in accordance with OECD TG 429 (GLP), with deviations: cell proliferation was measured by cell counting as opposed to radioactive labelling and included ear-swelling measurements. Comparing the acute reaction (ear weights) with the specific immune reaction (lymph node weights and cell counts) allowed for the distinction between the irritating potential and the sensitising potential of the test substance. Doses of 0 (control), 2, 10 and 50% prothioconazole (purity 97.2%) were administered to groups of six female NMRI mice. On day 4, the mice were sacrificed, the weights of the lymph nodes were measured, and the cell counts per mL of crushed lymph node were determined. The simulation index (calculated by dividing the lymph node weights and cell counts for exposed mice by that of the controls) are presented in Table 1.

Table 1*. Simulation indexes in a modified mouse LLNA with prothioconazole.*

Conc. (%)	Weight index (mean %)	Cell count index (mean %)
0	1.00	1.00
2	0.92	1.05
10	0.99	1.02
50	0.99	1.02

The criterion for a positive response with NMRI mice is a simulation index of ≥ 1.4. This was not reached in any dose group. The DS therefore considered prothioconazole not a skin sensitiser under the conditions of this study.

Based on two negative studies, the DS proposed no classification of prothioconazole for skin sensitisation.

Comments received during public consultation

One Member State Competent Authority (MSCA) asked if the modified LLNA using cell counting via flow cytometry, as opposed to measuring radioactively labelled nucleosides incorporated into dividing cells, had been validated and accepted. If not, classification for sensitisation should be considered based on the presence of two weakly positive skin sensitizers at ≤ 0.5% and ≤ 1.5%, respectively, in the technical mixture (purity 97.2%). The DS responded that a GPMT was first conducted on a high purity grade prothioconazole and was found to be negative. However, the mouse LLNA was performed in order to study the technical grade prothioconazole containing impurities with mild skin sensitizing properties. The DS pointed out that the modified procedure had been extensively studied and had been reported to have comparable sensitivity to the standard LLNA in the scientific literature. The performing laboratory regularly tested and

confirmed the reliability and sensitivity of the method. In addition, one of the impurities known to be a weak skin sensitiser was present at a level below the generic concentration limit for classification. The DS thus considered the LLNA test valid and that the negative results of the GPMT and LLNA showed that prothioconazole did not warrant a classification as a skin sensitiser.

Assessment and comparison with the classification criteria

Two skin sensitisation studies, one GPMT and one modified mouse LLNA, showed negative results for prothioconazole. For the modified mouse LLNA, a question was raised during the public consultation regarding the validity of the study. The modified protocol used cell counting via flow cytometry as opposed to measurement of incorporated radioactivity into DNA of dividing cells, as a proxy for cell counting. There is data from the performing laboratory to support the validity and sensitivity of the method, using alpha hexyl cinnamic aldehyde as a positive control (recommended in OECD TG 429) showing a clear positive response, and data to support that the method is comparable to the "standard" mouse LLNA (See: Vohr et al. 1994 and 2000; Ikarashi et al. 1993 and Homey et al. 1998).

RAC is therefore of the opinion that the modified protocol and the results can be considered reliable and valid. With regard to impurities with weakly sensitizing properties, the impurity present at ≤ 0.5% is below the generic concentration limit for classification whereas the other present at ≤ 1.5% could potentially be above. However, the technical mixture, including these two impurities, were tested in the mouse LLNA with a negative result. Altogether, based on negative results from the two reliable assays, RAC agrees with the DS that **no classification of prothioconazole for skin sensitisation is warranted**.

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

Summary of the Dossier Submitter's proposal

The DS presented studies on specific target-organ toxicity of prothioconazole upon repeated exposure in rats (oral and dermal 28-day, oral 90-day, 1-year and 2-year), mice (oral 90-day and 18-month), and dogs (oral 90-day and 1-year). The information (modified by the RAC) is summarized in Table 2 below.

Table 2. *Summary of repeated-dose toxicity studies on prothioconazole.*

Method, guideline, doses	Effects relevant for consideration of STOT RE.	CLP guideline values for classification (mg/kg bw/d)	DS evaluation and conclusion
Rat, 28-day dietary, OECD TG 407, GLP 0, 196, 1480, 9250 ppm ♂: 0, 18.6, 146, 952 mg/kg bw/d	952/1033 (♂/♀) mg/kg bw/d: ▪ ↓ bw gain (♂):-22% bw at 28d ▪ ↑ Rel. liver wt. (♂:+6%,♀:+23%**) ▪ ↑ ALT/ALP (♂:+48%*/+31%*, ♀:+39%*/+35%*) ▪ ↓ T4/↑ TSH (♂:-22%/+33%, ♀:-51%*/ +116%*) ▪ ↑ urea (♂: +22%, ♀: +27%) ▪ Pale, marbled kidneys with histological lesions ▪ ↑ Renal cell proliferation (♂,♀)	Cat 1 = 30 Cat 2 = 300	Effects in liver and kidney not adverse at dose levels relevant for STOT RE classification. Data not sufficient for classification.

Method, guideline, doses	Effects relevant for consideration of STOT RE.	CLP guideline values for classification (mg/kg bw/d)	DS evaluation and conclusion
♀: 0, 18.8, 151, 1033 mg/kg bw/d	<u>146/151 (♂/♀) mg/kg bw/d:</u> ▪ ↑ rel. liver wt. (♂: +4%, ♀: +6%) ▪ ↑ ALT/ALP (♂: +22%/+26%*, ♀: +10%/+30%*) <u>18.6/18.8 mg/kg bw/d</u> No treatment-related findings		
Rat, 28-day dietary and gavage, OECD TG 407, GLP Diet: 0, 10000 ppm (0, 1036 - 1066 mg/kg bw/d) 0, 10000 ppm (silica stabilized (SS)): 0, 1034 – 1082 mg/kg bw/d) Gavage: 0, 1000 mg/kg bw/d	<u>1036-1066 mg/kg bw/d (diet)</u> ▪ ↓ body weight gain (♂,♀) ▪ ↑ abs/rel. liver wt (♀:10-20%) ▪ Hepatocellular cytoplasmic change (♂,♀) ▪ Basophilic renal tubules (♂,♀) <u>1034-1082 mg/kg bw/d (SS diet)</u> ▪ ↓ body weight gain (♂,♀) <u>1000 mg/kg bw/d (gavage)</u> ▪ ↓ body weight gain (♂,♀) ▪ ↑ abs./rel. liver wt. (♀: 16%/17%) ▪ ↑ ALT (♂,♀), ↑ ALP (♀) ▪ Hepatocellular cytoplasmic change ▪ ↓ abs./rel. kidney wt. (♂: -13%/-8%) ▪ Basophilic renal tubules	Cat 1 = 30 Cat 2 = 300	Dosing above relevant guideline values. Data not suitable for classification.
Rat, 28-day dermal, OECD TG 410, GLP 0, 100, 300 and 1000 mg/kg bw/d	No adverse effects observed at any dose	Cat 1 = 60 Cat 2 = 600	No adverse effects at any dose. Data not sufficient for classification.
Rat, 90-day oral gavage, OECD TG 408, GLP 0, 20, 100, 500 mg/kg bw/d.	<u>500 mg/kg bw/day</u> ▪ ♀: 1/10 killed moribund, with necropsy findings in the kidney ▪ ↑ abs./rel. liver wt. (♂:+3%/ +6%, ♀:+12%*/+9%*) ▪ Hepatocellular hypertrophy (slight) and cytoplasmic change (♂:6/10, ♀:2/10) ▪ Minimal-slight basophilic renal tubules (♂: 9/10, Ctrl: 5/10) <u>100 mg/kg bw/day</u> ▪ Minimal-slight basophilic renal tubules (♂: 8/10, Ctrl: 5/10) <u>20 mg/kg bw/day</u> ▪ Minimal-slight basophilic renal tubules (♂: 8/10, Ctrl: 5/10)	Cat 1 = 10 Cat 2 = 100	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.

Method, guideline, doses	Effects relevant for consideration of STOT RE.	CLP guideline values for classification (mg/kg bw/d)	DS evaluation and conclusion
Rat, 90-day neurotoxicity study, Oral gavage, OECD TG 424, GLP. 0, 98, 505, 1030 mg/kg bw/d. 5 days/week.	<u>1030 mg/kg bw/day</u> ▪ ↓ body-weight gain (♂: -8.4%) ▪ ↓ motor activity (♂) ▪ ↓ locomotor activity (♂, ♀) <u>505 mg/kg bw/day</u> No relevant effects <u>98 mg/kg bw/day</u> No treatment-related effects	Cat 1 = 10 Cat 2 = 100	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.
Rat, 1-year, oral gavage, OECD TG 452, GLP 0, 5, 50, 750 mg/kg bw/d	<u>750 mg/kg bw/d</u> ▪ 3 deaths (no cause identified) ▪ ↓ bw gain (up 14% lower bw than controls at termination), ▪ ↑ rel. liver wt. (♂:+12%,♀:+29%**) ▪ Hepatocellular cytoplasmic change (♂, ♀) ▪ ↓ T3/T4 (♂:-8%*/-34%**, ♀:-3%/-35%*) ▪ ↑ kidney wt. (♂:+17%**, ♀:+10%**) ▪ ↑ Chronic progressive nephropathy (♂,♀) ▪ Urinary bladder: hyperplasia and focal inflammatory infiltration (♂, ♀) <u>50 mg/kg bw/day</u> No treatment-related findings <u>5 mg/kg bw/day</u> No treatment-related findings	Cat 1 = 2.5 Cat 2 = 25	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.
Rat, 2-year, oral gavage, OECD TG 451, GLP 0, 5, 50, 750 mg/kg bw/d. 750 mg/kg bw/d reduced to 500 mg/kg bw/d (week 84, males) and 625 mg/kg bw/d (week 56, females)	<u>750/500, 750/625 mg/kg bw/d</u> ▪ ↑ mortality (26% survival at termination in males) ▪ ↓ bw gain (up to 20% lower bw than controls at termination) ▪ ↑ rel. liver wt (♂:+25%**, ♀:+26%**) ▪ Hepatocellular hypertrophy with cytoplasmic change eosinophilic/clear cell foci with cytoplasmic change (♂, ♀) ▪ ↑ ALP (♂:+38%*, ♀:+73%*) ▪ ↓ T4 (♂:-59%**, ♀:-31%**) ▪ ↑ rel. kidney wt. (♂:+30%**, ♀: +11%*) ▪ ↑ Chronic progressive nephropathy (♂,♀) ▪ yellow brown crystalloid structures in urine sediment ▪ Urinary bladder: hyperplasia and inflammation (♂, ♀) <u>50 mg/kg bw/day</u> ▪ Hepatocellular hypertrophy with cytoplasmic change (♂) ▪ ↓ T4 (♂:-26%**, ♀:-12%*) ▪ ↑ Chronic progressive nephropathy (♂,♀) <u>5 mg/kg bw/day</u>	Cat 1 = 1.25 Cat 2 = 12.5	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.

Method, guideline, doses	Effects relevant for consideration of STOT RE.	CLP guideline values for classification (mg/kg bw/d)	DS evaluation and conclusion
	No findings		
Mouse, 90-day oral gavage, OECD TG 408. GLP. 0, 25, 100, 400 mg/kg bw/d.	<u>400 mg/kg bw/day</u> ▪ ↑ cholesterol (♀:+41%**) ▪ ↓ bilirubin (♂:-42%**, ♀:-28%**) ▪ ↑ hepatic enzyme activity (♂,♀) ▪ ↑ abs/rel liver wt. (♂:+44%**/ +56%**, ♀:+39%**/+37%**) ▪ Hepatocellular hypertrophy (♂: 9/10, ♀: 10/10) ▪ Centrilobular/periportal fatty change (♂: slight-moderate, ♀: minimal-slight) ▪ Hepatocellular focal necrosis <u>100 mg/kg bw/day</u> ▪ ↑ abs/rel liver wt. (♂:+13%/+ 21%**, ♀: +14%/+15%*) ▪ Hepatocellular hypertrophy (♂: 9/10, ♀:3/10) ▪ Centrilobular fatty change (♂: minimal, ♀: minimal-slight) <u>25 mg/kg bw/day</u> No adverse effects	Cat 1 = 10 Cat 2 = 100	Effects in liver and kidney not adverse at dose levels relevant for STOT RE classification. Data not sufficient for classification.
Mouse, 18 months, oral gavage, OECD TG 451, GLP. 0, 10, 70 and 500 mg/kg bw/d	<u>500 mg/kg bw/day</u> ▪ ↓ bw gain (♂: -9%, ♀: -13% bw vs controls at termination) ▪ ↑ abs/rel liver wt. (♂:+25%**/+39%** ,♀:+21%**/+39%**) ▪ Hepatocellular hypertrophy with cytoplasmic change (♂, ♀) ▪ ↓ abs/rel kidney wt (♂: -20%**/-13%**, ♀: -15%**/-) ▪ Renal tubular degeneration/ regeneration, subcapsular tubular degeneration/fibrosis (♂, ♀) <u>70 mg/kg bw/day</u> ▪ ↓ bw gain (♂: -3%, ♀: -7% bw vs controls at termination) ▪ ↑ abs/rel liver wt. (♂: +12%**/+16%**, ♀: +4%**/+10%**) ▪ Hepatocellular hypertrophy with cytoplasmic change (♂) ▪ Renal tubular degeneration/regeneration (♂) <u>10 mg/kg bw/day</u> No treatment-related effects	Cat 1 = 1.7 Cat 2 = 17	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.
Dog, 90-day oral gavage, OECD TG 409, GLP	<u>300 mg/kg bw/day</u> ▪ ↑ rel. liver wt. (♂: +20%, ♀: +19%*) ▪ ↑ hepatic microsomal enzymes (2 fold, ♀) ▪ ↑ ALT (♂: +25%, ♀: +113%*) ▪ ↑ ALP (♂: +72%, ♀: +41%)	Cat 1 = 10 Cat 2 = 100	Effects in liver and kidney not adverse at dose levels relevant for STOT RE classification.

Method, guideline, doses	Effects relevant for consideration of STOT RE.	CLP guideline values for classification (mg/kg bw/d)	DS evaluation and conclusion
0, 25, 100, 300 mg/kg bw/day, 5 days/week. 8 week recovery period (control and high dose groups).	▪ ↓ T4 (♂: -39%, ♀: -44%*) ▪ ↑ kidney wt. (♂: +16%, ♀: +18%*) ▪ Kidney cysts (♂: 1/4 main group, 1/4 recovery group) ▪ Renal histopathology: chronic inflammation (slight), multi-focal chronic interstitial fibrosis in cortex and medulla, crystalline material in two males (minimal-slight) ▪ Tubular degeneration (♂) <u>100 mg/kg bw/day</u> ▪ ↑ ALT (♀: +28%) ▪ ↑ ALP (♂: +60%, ♀: +11%) ▪ ↑ hepatic microsomal enzymes (2 fold, ♀) ▪ ↑ liver wt. (♀: +11%) ▪ Renal histopathology: chronic inflammation (slight), multi-focal chronic interstitial fibrosis in cortex and medulla, crystalline material in one male (slight) <u>25 mg/kg bw/day</u> No adverse effects		Data not sufficient for classification.
Dog, 1-year, oral gavage, OECD TG 452, GLP 0, 5, 40, 125 mg/kg bw/d, 5 days/week	<u>125 mg/kg bw/d</u> ▪ ↓ bw gain/↓ terminal bw (♂:-14%/-3%, ♀:-42%/-15%) ▪ ↑ abs/rel liver wt. (♂:+20%/+23%*, ♀: +8%/+35%*) ▪ ↑ CYP450 (♂: 2-fold, ♀: 3-fold) ▪ Liver pigmentation (♂,♀) ▪ ↑ ALP (♂:+13%, ♀:+148%*) ▪ ↑ abs/rel kidney wt (♀: +5%/ +31%*) ▪ Chronic renal inflammation, crystalline material in tubules, pigmentation (♂,♀) <u>40 mg/kg bw/d</u> ▪ ↓ Bw gain/↓ terminal bw (♂: -11%/-3%) ▪ ↑ ALP (♀: +8%) ▪ ↑ CYP450 (♀: 2.5-fold) ▪ Chronic renal inflammation, pigmentation <u>5 mg/kg bw/d</u> No adverse effects	Cat 1 = 2.5 Cat 2 = 25	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.

* Significantly different from control, $p \leq 0.05$, ** Significantly different from control, $p \leq 0.01$

The liver and the kidneys were identified as the relevant target organs following repeated oral exposure to prothioconazole.

The effects on the liver were indicative of adaptive responses to hepatic metabolism of prothioconazole and consisted of increased absolute and relative liver weights, hepatic enzyme induction (incl. clinical markers of liver injury) and hepatocellular hypertrophy with cytoplasmic changes. Although some effects were substantial at the higher doses, the effects at doses relevant for classification were relatively small and not associated with adverse histopathological

changes and were therefore regarded by the DS as adaptive rather than adverse. Prolonged administration of prothioconazole for 18 months at doses up to 500 mg/kg bw/d did not exacerbate the hepatotoxicity and no necrosis or fatty change was observed. The DS therefore did consider the effects in liver observed at dose levels relevant for classification not adverse and insufficient for classification.

Effects on the kidneys comprised changes in urinary output, biochemical alterations and pathological findings. Histopathological changes consisted of an increased incidence and severity of basophilic tubules and tubular dilatation in rats in the 28-day and 90-day studies while severe toxicity, including deaths, was observed at the high dose of 750 mg/kg bw/d in the 1-year study. In dogs, chronic inflammatory changes (interstitial fibrosis and inflammation) were observed in the 90-day study (males only) from the mid dose of 100 mg/kg bw/day. The inflammatory changes of slight severity were characterized by multi-focal chronic interstitial fibrosis in the cortex and medulla, inflammation (minimal) and debris (crystalline material) in one male. There was no renal proximal tubular epithelial cell degeneration at this dose. At the next dose (300 mg/kg bw/d; above the guidance value for classification), the inflammatory effects progressed slightly in severity and were associated with increased organ weight and proximal tubular epithelial degeneration. There were no adverse effects at the low dose level of 25 mg/kg bw/d. Following dosing up to 125 mg/kg bw/d in dogs for one year, renal chronic inflammation and pigmentation were noted in males at 40 mg/kg bw/d. Although there was not a dose-related increase in the incidence of chronic inflammation, the severity score of the pigmentation at 125 mg/kg bw/d was lower than that at the mid dose level (40 mg/kg bw/d). The DS therefore considered it uncertain whether these effects were related to the treatment. Altogether, the DS considered the kidney as a clear target organ for prothioconazole following repeated oral exposure. However, in most studies the renal toxicity was only evident at doses exceeding the cut-off values for category 2. In one study, the 90-day study in dogs, effects (graded as minimal to slight) were observed at a dose relevant for classification. Extension of the dosing period to one year did not increase the incidence or severity of renal findings. Therefore, the DS did not consider the renal effects sufficient for STOT-RE classification.

Altogether, based on lack of adverse effects at levels relevant for classification the DS proposes no classification for STOT RE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

In total 11 reliable OECD TG compliant repeated-dose toxicity studies (GLP) in rats, mice and dogs were presented. The liver, kidney and urinary tract were clearly the target organs of prothioconazole.

Liver effects were indicative of responses to extensive hepatic metabolism of the substance - enzyme induction, hepatocellular hypertrophy with cytoplasmic changes, increased liver weight and slight increases in clinical markers of hepatocellular injury (ALT/ALP). Hepatocellular necrosis was observed only at the top dose in one study. At dose levels relevant for classification, effects on the liver were observed in the subacute and subchronic studies. However, these effects can be considered adaptive and not of sufficient adversity to warrant classification. No degenerative changes were observed at these dose levels. In addition, no treatment-related effects were observed in the longer-duration chronic studies at levels relevant for classification. RAC therefore

agrees with the DS that the effects on the liver of prothioconazole are not sufficient for classification.

Effects on circulating thyroid hormones were observed in some studies above dose levels relevant for classification, but they were not connected to any effects on thyroid weight or histopathology and were likely due to the extensive hepatic metabolism and increased hormonal clearance.

Kidney effects consisted of inflammatory changes (inflammation, interstitial fibrosis), basophilic tubules, tubular dilation, tubular pigmentation and tubular degeneration and regeneration. Crystalline material in tubules were observed at higher doses. At dose levels relevant for classification, effects on the kidneys consisted of minimal-slight basophilic tubules in rats (90-day) and histopathological changes in dogs (90-day): chronic inflammation (slight) and focal chronic interstitial fibrosis in cortex and medulla. At the dose level relevant for classification in the 90-day dog study, 100 mg/kg bw/d, chronic inflammation/ interstitial fibrosis occurred in 3/4 males and 1/4 females (Table 3). Identical incidences were reported at 300 mg/kg bw/d, however with a slightly higher severity grade in males and lower in females. The effects at 300 mg/kg bw/d were partially reversed following an 8-week recovery period (not examined at 100 mg/kg bw/d). Multifocal fibrosis, which is not expected to be reversible, could warrant classification. However, in the 1-year dog study, no fibrosis was observed in males up to 125 mg/kg bw/d, and it was noted in 1/4 females (graded as minimal) at 125 mg/kg bw/d (Table 4). It is therefore clear that the results from the 90-day study were not replicated in the 1-year study and that the effects did not worsen despite the longer study duration. There were also not clear dose-responses in the studies with regard to chronic inflammation/fibrosis. Altogether, RAC considers these effects on the kidney at dose levels relevant for classification not sufficient for classification and therefore agrees with the DS.

Table 3. *Incidence and severity of histopathological findings in kidneys of dogs after 90-day exposure to prothioconazole with/without recovery period (RAR Table B.6.3.23).*

Parameter	No. animals affected (mean severity*) in:							
	Males (mg/kg bw/d):				Females (mg/kg bw/d):			
	0	25	100	300	0	25	100	300
Kidneys (no examined)	4	4	4	4	4	4	4	4
- cyst	0	0	0	1 (2.0)	0	0	0	0
- degeneration	0	0	0	3 (2.0)	0	0	0	0
- inflammation	0	0	0	0	0	0	1 (1.0)	0
- inflammation, acute	0	0	1 (1.0)	1 (1.0)	0	0	0	0
- inflammation, chronic	1 (1.0)	0	3 (2.0)	3 (2.3)	0	0	1 (3.0)	1 (1.0)
- mineralization	0	2 (2.0)	0	0	1 (1.0)	0	0	0
- debris	0	0	1 (2.0)	2 (1.5)	0	0	2 (2.0)	0
- lipidosis, glomerular	1 (1.0)	2 (1.0)	0	1 (1.0)	0	0	0	0
Recovery groups								
Kidneys (no examined)	4	0	0	4	4	0	0	3
- cyst	0	-	-	1 (2.0)	0	-	-	0
- degeneration	0	-	-	0	0	-	-	1 (3.0)
- inflammation	0	-	-	0	0	-	-	0
- inflammation, acute	0	-	-	0	0	-	-	0
- inflammation, chronic	0	-	-	2 (2.0)	0	-	-	1 (2.0)
- mineralization	0	-	-	0	0	-	-	0
- debris	0	-	-	0	0	-	-	0
- lipidosis, glomerular	0	-	-	0	1 (1.0)	-	-	0

*Severity was graded from 1 (minimal) to 5 (severe)

Table 4. *Incidence and severity of histopathological findings in kidneys of dogs after 1-year exposure to prothioconazole (RAR Table B.6.3.27).*

Parameter	No. animals affected (mean severity*) in:							
	Males (mg/kg bw/d):				Females (mg/kg bw/d):			
	0	**5**	**40**	**125**	**0**	**5**	**40**	**125**
Kidneys (no examined)	4	4	4	4	4	4	4	4
- crystals	0	0	0	1 (1.0)	0	0	1 (1.0)	2 (1.0)
- cyst	1 (1.0)	1 (1.0)	0	0	0	0	0	0
- fibrosis	0	0	0	0	0	0	0	1 (1.0)
- hyperplasia	0	0	0	0	0	1 (1.0)	0	0
- inflammation, chronic	0	0	1 (2.0)	1 (3.0)	1 (2.0)	0	0	4 (1.5)
- inflammation, chronic active	0	0	1 (1.0)	0	1 (1.0)	0	0	0
- mineralization	0	0	0	1 (1.0)	1 (1.0)	1 (1.0)	0	0
- pigmentation	0	0	1 (3.0)	2 (1.5)	1 (1.0)	0	1 (2.0)	0
- lipidosis, glomerular	0	0	0	0	1 (1.0)	0	0	0
- mineralisation, pelvis	0	1 (1.0)	0	0	0	1 (1.0)	0	0

*Severity was graded from 1 (minimal) to 5 (severe)

In addition to effects on the kidney, effects were also observed in the urinary bladder (inflammation, hyperplasia), however, at dose levels not relevant for classification.

RAC agrees with the DS that **no classification for STOT RE for prothioconazole is warranted**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

For the evaluation of germ cell mutagenicity of prothioconazole, the DS presented results from five *in vitro* studies, covering bacterial- and mammalian-cell mutation, clastogenicity and aneugenicity (Table 5), and in three *in vivo* assays, one unscheduled DNA synthesis assay in rat liver and two mouse micronucleus tests (Table 6).

Table 5. *Results of genotoxicity tests of prothioconazole in vitro.*

Method, guideline	**prothioconazole purity (%)**	**Result**
Ames test, OECD TG 471 (1997), GLP	99.5	Negative (± S9)
Mammalian cell mutation assay (V79-HPRT), OECD TG 476 (1984, 1997), GLP	99.8	Negative (± S9)
Mammalian chromosome aberration test, OECD TG 473 (1983), GLP	99.8	Positive (± S9). However, cytotoxic concentrations used.
In vitro micronucleus assay in human lymphocytes, OECD TG 487 (2016), GLP	97.6	Negative
Unscheduled DNA synthesis assay, OECD TG 482 (1986), GLP	99.7	Negative

Table 6. *Results of genotoxicity tests of prothioconazole in vivo.*

Method, guideline, test animal/strain	prothioconazole purity (%)	Result
Unscheduled DNA synthesis assay, OECD TG 486 (1997), GLP, Wistar rats	99.5-99.7	Negative
Micronucleus assay (*in vivo* mouse bone marrow), OECD TG 474 (1983), GLP, NMRI mice	99.7	Negative
Micronucleus assay (*in vivo* mouse bone marrow), OECD TG 474 (1997), GLP, NMRI mice	99.7	Negative

Overall, prothioconazole showed negative results in 4/5 assays *in vitro* and in 3/3 assays *in vivo*. One assay showed positive results, however only at concentrations resulting in excessive cytotoxicity. The DS therefore proposed no classification of prothioconazole for germ cell mutagenicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Prothioconazole was negative in all tested assays *in vitro* and *in vivo*, except in one assay *in vitro* at high cytotoxic concentrations. RAC therefore agrees with the DS that **no classification for germ cell mutagenicity is warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

For carcinogenicity, the DS presented two long-term toxicity/carcinogenicity studies in rats (1- and 2-year studies) and an 18-month carcinogenicity study in mice.

In the 1-year chronic toxicity study (OECD TG 452, GLP), prothioconazole was dosed by gavage at 0, 5, 50 and 750 mg/kg bw/d to Wistar rats (20/sex/group). Liver and kidney/urinary tract were identified as target organs/tissues (for details of non-neoplastic findings see Table 2). Five animals at 750 mg/kg bw/d were found dead or sacrificed moribund. Two deaths were attributed to gavage errors, but the study authors did not identify the cause of the other deaths. The applicant suspected these to be due to kidney toxicity at this dose. No treatment-related increase in tumours was found in this study.

In the 2-year carcinogenicity study (OECD TG 451, GLP), prothioconazole was dosed by gavage at the same doses as in the 1-year study: 0, 5, 50 and 750 mg/kg bw/d to Wistar rats (50/sex/ group). The longer duration of the study led to an exceedance of the maximum tolerated dose (MTD) at 750 mg/kg bw/d, with increased number of deaths and emaciation. This dose was therefore reduced during the study to 500 mg/kg bw/d in males from week 84 and to 625 mg/kg bw/d in females from week 56. The increased number of deaths in the males resulted in a survival of <50% in the high dose group at study termination (26% survival). The mortality rates in females at termination were similar to controls. Liver and kidney were identified as target organs (for details of non-neoplastic findings see Table 2). No increase in tumours in any other organ or tissue was observed. The DS speculated whether the low survival in the high dose males could

have reduced the sensitivity of the study. However, survival in this group was reduced by more than 50% only at a late time point of the study (at around 22 months) and all animals were examined for tumours. There was no indication of an increase in tumour incidences or of a decreased tumour latency in decedents or animals that survived to termination. The DS considered the presence of toxicity in mid dose males, without neoplastic findings, to provide reassurance that the study was not compromised by the reduced survival of the high dose males. Furthermore, survival of the high dose female group was satisfactory, not only above 50% but similar to the control. The DS therefore concluded that the study was adequate for the detection of a carcinogenic potential of prothioconazole.

In the 18 months carcinogenicity study (OECD TG 451, GLP), prothioconazole was dosed via gavage at the doses 0, 70, 70 and 500 mg/kg bw/d to CD-1 mice (60/sex/dose). Survival was similar between all groups at termination, apart from a slightly higher mortality rate in the top dose males. As compared to controls the terminal body weights were decreased by -9% and -13% in males and females at 500 mg/kg bw/d, respectively, and clinical signs at the top dose consisted of piloerection and poor general condition. The liver and kidney were identified as the target organs (for details on the non-neoplastic findings see Table 2 in the STOT RE section). There were no increases in tumour incidences in the liver, kidney or any other organ/tissue in any dose group. The total number of tumours was lower in the high dose groups than in the controls. The DS thus concluded that prothioconazole was not carcinogenic in mice in this study. The DS considered that the combination of the body weight effects and the high incidence of histopathological findings in the kidney suggest that the high dose level reached the MTD and was thus sufficiently high to conform with the guideline requirements.

Altogether, based on the three available studies, not showing any increases in tumours in the liver, kidney or any other organ, the DS concluded that no classification for carcinogenicity for prothioconazole was warranted.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The DS presented the results of three reliable chronic toxicity/carcinogenicity studies in rats and mice, performed in accordance with OECD TGs and GLP. None of the studies showed any indication of increased tumour incidence. In the 1-year and the 2-year rat studies, mortality occurred at the top dose, showing that the MTD was reached. In the 2-year study, despite a lowering of the top dose, mortality in the males was significantly higher in the top dose as compared to the control. The mortalities, however, occurred at a late time point and all animals were investigated for tumours, showing negative results. Altogether, the outcome of these studies show that the MTD was reached and that no increase in tumours occurred. In the 18 months mouse study, general toxicity at the high dose consisted of a decreased body weight gain of 39% in both males and females with a 9% and 13 % lower terminal body weight as compared to controls in males and females, respectively, despite normal food consumption. Clinical signs consisted of piloerection, pallor and general poor condition occurring at the end of the study. The decreased body weight gain and clinical symptoms at the top dose showed that the MTD was reached in this study.

Based on three negative and valid chronic toxicity/carcinogenicity studies, supported by a lack of genotoxicity, RAC agrees with the DS that **no classification of prothioconazole for carcinogenicity is warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of prothioconazole has been studied in rats and rabbits. For effects on sexual function and fertility, the DS presented the results of one two-generation study in rats, supplemented with a range-finding study. For developmental toxicity, three developmental toxicity studies in rats (two oral and one dermal) and one in rabbits (oral) were presented with associated range-finding studies for the oral studies.

Sexual function and fertility

In the dose-range finding study (non-guideline, GLP), prothioconazole (purity 98.1-98.8%) was dosed by gavage at 0, 10, 100, 250 and 500 mg/kg bw/d in Hanover Crl:WI(Han) Wistar rats (10/sex/dose) from 4 weeks pre-mating until postnatal day (PND) 21. Effects were only seen at the top dose where parental males showed slightly lower body weight gains and terminal body weights than controls. Pups showed 7% lower body weights than controls at PND21. No effects on maternal body weights or on parameters on sexual function and fertility were noted. The only potential treatment-related clinical signs were urine stained fur in both sexes at the top dose, interpreted by the DS as being related to kidney toxicity. The study authors concluded that the top dose in the two-generation study should be between 500 and 1000 mg/kg bw/d.

In the two-generation study (OECD TG 416, GLP), prothioconazole (purity 98.1-98.8%) was given via gavage to groups of 30 Hanover Crl:WI(Han) Wistar rats/sex at 0, 10, 100 and 750 mg/kg bw/d from pre-mating until weaning of F1 pups. Selected F1 progeny received the same doses until weaning of the F2 generation. Parental toxicity was observed at 750 mg/kg bw/d and consisted of slightly decreased body weight gains of up to 5% in dams during gestation of both generations. Clinical signs in both generations included urine staining, salivation and dehydration, indicative of kidney dysfunction. The efficiency of food utilisation in the high dose P-generation males was decreased, demonstrated by an increased food consumption (up to 19%) with a co-occurring reduction in body-weight gain of ≤7% compared to controls. In the F1-generation, high dose animals showed lower initial body-weights than controls (♂: -17%, ♀: - 7%), a difference that was maintained throughout the F1 pre-mating period despite increased food consumption of up to 28% over this period. No relevant general toxicity or clinical signs were evident at 10 or 100 mg/kg bw/d.

Mating, fertility and gestation indices were unaffected at all dose-levels in both generations. Slight, statistically non-significant, increases in gestation length and reduced numbers of implantation sites and litter sizes were seen in both generations at the high dose. In the F1-generation, increases in the number of days to insemination were also observed. There were decreases in the number of oestrus cycles and increases in cycle length at 750 mg/kg bw/d, being more pronounced in the F1 generation. These were considered by the DS secondary to maternal toxicity and not relevant for classification. An examination of primordial ovarian follicles and corpora lutea counts did not reveal any dose-response relationship. There were no treatment-related changes in sperm parameters. All the parameters relevant for sexual function and fertility were unchanged in the mid and low dose groups.

Parameters of pup viability (birth index, live-birth index, viability index, lactation index) were unaffected by administration of prothioconazole at all doses. Toxicity in the offspring was observed in the high dose group as decreased body weight gain. At PND21, body weights were -16%/-14% and -9%/-7% as compared to controls in F1 and F2 males/females, respectively. A slight delay in preputial separation in F1 pups occurred at 750 mg/kg bw/d, and was considered by the study authors and DS to be a result of the retarded growth. An analysis of the body weight of the pups on the individual day of preputial separation showed an association between body

weight and the day of preputial separation. A slightly greater anogenital distance was found in F2 male and female high dose pups at birth (within HCD range). It was correlated with a higher body weight at birth, which the study authors and the DS attributed to a slightly longer duration of gestation of the respective dams.

Altogether, the DS concluded that prothioconazole did not demonstrate a specific effect on sexual function and fertility. Some changes occurred in the high dose group, but these were considered by the DS to be secondary to relatively severe maternal toxicity. Therefore, the DS proposed no classification for prothioconazole for effects on sexual function and fertility.

Development

Developmental toxicity studies in rats

In the first dose-range finding study (non-guideline, non-GLP), female Wistar rats (5/dose, sub-strain not specified) were given prothioconazole (purity not specified) by oral gavage at 100, 300 and 1000 mg/kg bw/d, GD 6-19. There were no treatment-related deaths or adverse clinical signs at any dose. Slight initial reduced body-weight gain was reported at 300 and 1000 mg/kg bw/d. Food consumption was unaffected at all dose levels. Increased water consumption and urination occurred in the top dose. There were no treatment-related external abnormalities detected at any dose. Non-dose-related increased incidences of supernumerary ribs occurred at 300 mg/kg bw/d (24% of foetuses with 14th or cervical ribs) and 1000 mg/kg bw/d (12% of foetuses with 14th ribs) but not at 100 mg/kg bw/d. No skeletal abnormalities were detected during a brief examination of the specimens.

In the main developmental toxicity study (OECD TG 414, GLP), prothioconazole (99.5-99.8% purity) was administered by oral gavage to female Wistar Hsd Cpb:WU rats (26/group/dose) at 0, 80, 500 and 1000 mg/kg bw/d during GD 6-19. No treatment-related deaths occurred. Maternal toxicity occurred at the mid and top dose and consisted of increased water consumption (59-75%) and increased urination. Food consumption was reduced at the top dose between GD 6-11. Body weight loss occurred at GD 6-8 in the high dose group (-1.4%) and cumulative body-weight gain was decreased at the mid and high dose during gestation (corrected bw. gain -21% and -31%, respectively, compared to control). Pregnancy incidences, the mean number of corpora lutea, and implantations were similar across all groups. Live litter size, placental weight and foetal sex ratios were unaffected at all dose levels. Foetal body weights were slightly lower at 1000 mg/kg bw/d in both sexes (-4% to -5% as compared to controls). There was an increased incidence of microphthalmia in all prothioconazole-exposed groups compared with the concurrent control. At 80 and 500 mg/kg bw/d, the incidences did not show a dose-related increase and they were on a litter basis within the HCD range and individually outside the HCD range at 80 mg/kg bw/d but not at 500 mg/kg bw/d (see Table 7 below). The incidence of microphthalmia in the high dose group was outside the range of HCD and included two foetuses with bilateral microphthalmia (considered more likely to indicate an effect of treatment) which was not observed in the other groups.

Table 7. *Incidences (%) of microphthalmia – foetal (litter).*

Dose (mg/kg bw/d)	0	80	500	1000	HCD range[a]
Microphtalmia	0 (0.0)	2.4 (15.4)	1.1 (13.6)	4.6 (33.3)	0-1.95 (0-20)

[a] Data from 1993-99 (26 studies)

Previous studies in the same laboratory have demonstrated a high variability with regard to microphthalmia in this strain of rats (Table 8). With support of that variability, the DS concluded that the absence of a dose-response relationship and incidences within the HCD range, the cases of microphthalmia at 80 and 500 mg/kg bw/d were incidental and not treatment-related, however that the effects at 1000 mg/kg bw/d were treatment-related.

Table 8. *Examples of inter group variability of microphthalmia.*

Year	Study	Litter incidence of microphthalmia (%)			
		Control	Low dose	Mid dose	High dose
1995	T2055246	17.9	6.5	6.3	17.2
1996	**prothioconazole**	0	15.4	13.6	33.3
1997	T0060860	20.0	0	4.2	27.8
2002	T6071558	20.0	12.5	4.8	0

For 1000 mg/kg bw/d, the DS grouped maternal and foetal data for dams that produced pups with microphthalmia and for those who did not (Table 9). The grouping showed that maternal toxicity was present in all dams at 1000 mg/kg bw/d, but more pronounced with regard to reduced maternal body-weight gain in dams that gave birth to foetuses with microphthalmia. Foetal weight was generally reduced at this dose but the reduction was more pronounced in litters including foetuses with microphthalmia. The DS therefore concluded, based on these correlations that this effect was non-specific and secondary to maternal toxicity.

Table 9. *Mean values (percentages) for maternal body weight gain and feed intake and foetal weights at 1000 mg/kg bw/d, grouped for dams that delivered pups with microphthalmia (+MO) and for those with pups without microphthalmia (-MO) in comparison to control.*

Group	Maternal b.w. change		Corrected mat. b.w. change GD 0-20	Feed intake GD 6-11 (g/animal/d)	Live foetal b. w.
	GD 6-8	GD 6-11			
1000 mg/kg +MO	**-5.4 g** (-204%)	**+4.3 g** (28%)	**+21.1 g** (53%)	**13.4** (72%)	**3.38 g** (93%)
1000 mg/kg -MO	**-1.4 g** (-127%)	**+8.1 g** (52%)	**+30.8 g** (77%)	**16.3** (88%)	**3.48 g** (96%)
Control	**+5.2 g**	**+15.5 g**	**+40.0 g**	**18.6**	**3.63 g**

The only treatment-related visceral finding observed apart from microphthalmia was dilatation of the renal pelvis, recorded at a higher incidence in animals at 1000 mg/kg bw/d. The study authors and the DS considered this secondary to delayed foetal development (as indicated by decreased foetal body weight at this dose).

Treatment-related skeletal findings consisted of a dose-related increase in rudimentary supernumerary (punctiform and comma-shaped) 14th ribs (Table 10) and decreases in ossification at 1000 mg/kg bw/d. Decreased ossification outside the HCD range (distal and proximal phalanges, caudal vertebral bodies and 6th sternebral bone) were recorded only in the high dose group. The DS considered this effect secondary to maternal toxicity and the delayed foetal development.

Table 10. *Incidence of rudimentary ribs (foetal (litter) incidence (%))*

Dose (mg/kg bw/d)	0	80	500	1000	HCD range[a]	HCD range[b]
Short 14th rib	0.7 (3.8)	7.1* (42.3)**	10.6* (54.5)**	25.2** (62.5)**	0-12.2 (0-40)	0-24.4 (0-57)

[a] Data from 1993-1999 (24 studies)

[b] Data from 1992-2000 (29 studies: 24 studies as above + 5 studies from 1992 and 2000)

The same variation, rudimentary supernumerary 14th ribs, was found in the range-finding study at 300 and 1000 mg/kg bw/d, without a dose-response relationship. No fully formed 14th ribs (considered a variation) were observed at any dose in the range finding or the main study. HCD from the same laboratory and strain showed that rudimentary 14th ribs commonly occur spontaneously in untreated rats, reported in all but one of the 53 historical studies. However, this was not reflected in the control of the present study; the control incidence for rudimentary 14th ribs was unusually low, being the lowest of 52 studies. Foetal and litter incidences were within these historical control ranges up to and including 500 mg/kg bw/d and slightly above at

1000 mg/kg bw/d. The DS therefore concluded that the increase in this variation at 1000 mg/kg bw/d was treatment-related but secondary to maternal toxicity.

To follow up on whether the increase in microphthalmia could be related to maternal toxicity, the applicant provided information on another substance, the respiratory tract irritant cyfluthrin, tested in the same strain of rats as prothioconazole. Female Wistar Hsd Cpb:WU rats were exposed by inhalation to cyfluthrin at doses of up to 3 mg/kg bw/d; with one extra control and the high dose groups also receiving supplementary oxygen. Clinical signs of toxicity were apparent in the dams at dose levels ≥ 1.0 mg/kg bw/d and an increased incidence of microphthalmia occurred from that dose (Table 11). Oxygen supplementation reduced the maternal toxicity as well as the incidences of foetuses with microphthalmia. In addition, the results of an oral study with cyfluthrin up to a dose of 30 mg/kg bw/d (10x higher than via inhalation) was presented, that did not result in embryotoxicity or any cases of microphthalmia, that was suggested to rule out a teratogenic potential of cyfluthrin. Altogether, the applicant and the DS considered that these results together indicated that the cases of microphthalmia correlated well with maternal toxicity and that the prothioconazole-induced cases of microphthalmia in this strain of rat were secondary to maternal toxicity.

Table 11. *Incidences of microphthalmia (%) in Wistar Hsd Cpb:WU rats following maternal inhalation exposure to cyfluthrin.*

	Cyfluthrin (mg/kg bw/d)					
	Ctrl.	**Ctrl + oxygen**	**0.2**	**1.0**	**3.0**	**3.0 + oxygen**
Microphtalmia (%)	0.76	0.41	0.41	1.20	5.4	2.9

To follow up on the findings of microphthalmia caused by prothioconazole, a set of supplementary studies was provided: a dose-range finding study and a full developmental toxicity study in another strain of Wistar rats, Hanover Crl:WI(Han). This strain has a very low background incidence of microphtalamia, but with demonstrated sensitivity to this malformation based on studies with the teratogen all-trans retinoic acid.

In the range-finding study (non-guideline, GLP), female rats (12/group) were dosed with prothioconazole (98.7% purity) at 0, 500 and 1000 mg/kg bw/d during GD6-19. Dehydration occurred in one dam at 500 mg/kg bw/d and in three dams (which died) at 1000 mg/kg bw/d. There were no external malformation in pups found at any dose. Based on the mortality at 1000 mg/kg bw/d, the top dose in the main study was set to 750 mg/kg bw/d.

In the main developmental toxicity study (OECD TG 414, GLP), in Hanover Crl:WI(Han) Wistar rats, female rats (25/group) were exposed to prothioconazole at 0, 20, 80 and 750 mg/kg bw/d during GD6-19. There were no deaths or treatment-related clinical signs of toxicity at any dose. At 750 mg/kg bw/d, the overall body-weight gain was reduced by 46% (corrected bw gain reduced by 13%) during GD6-12. Food consumption was decreased by up to 27% on GD6-12. Water consumption was increased on days 11-20 by up to 74% and clinical chemistry parameters indicated functional impairments of kidneys and liver. There were no treatment-related general toxicity in the low and mid dose groups.

No reproductive effects, such as differences in litter size, sex ratio, foetal or placental weights were detected in any group. The ocular external examinations did not show any foetus with microphthalmia in any dose group. No differences were observed on the individual or mean eye weights, eye-to-foetal weight ratios, or on eye measurements. The samples collected showed normal distribution pattern of eye weight in the control and high dose foetuses. The DS therefore concluded that prothioconazole did not cause microphthalmia in this study. The skeletal evaluation showed a possible treatment-related increase in supernumerary rudimentary ribs at 750 mg/kg bw/d. A treatment-related effect on fully formed supernumerary ribs was not found. The foetal incidence of comma-shaped rudimentary ribs was marginally outside the HCD range

for the same laboratory and rat strain and that of the punctiform ribs was well within the upper boundary of the HCD range (Table 12).

Table 12. *Supernumerary 14th ribs (foetal (litter) incidence (%))*

Dose (mg/kg bw/d)	0	20	80	750	HCD range[a]
Rudimentary (punctiform)	23.5 (95.2)	18.2 (77.8)	27.6 (88.9)	33.6 (95.7)	19 – 52 (57 - 91)
Rudimentary (comma-shaped)	11.8 (52.4)	7.4 (66.7)	12.4 (38.9)	21.2*(69.6)	5 – 18 (9 - 58)

[a] Data from 4 studies (522 foetuses, 97 litters) conducted 1998-2002, same laboratory and strain, *p ≤ 0.05, ** p ≤ 0.01

The DS considered this study to show that prothioconazole did not cause microphthalmia even when it was tested up to doses that were severely toxic to the dams. The marginal increase in rudimentary ribs outside HCD range was not considered by the DS to be an adverse effect but a common variation associated with maternal stress.

In the dermal developmental toxicity study (OECD TG 414, GLP, Anon., 2001f), dermal administration of prothioconazole to female Wistar rats (29-30/group) at doses up to 1000 mg/kg bw/d did not result in any adverse effect at any dose.

Developmental toxicity studies in rabbits

In an oral range finding study in rabbits (non-guideline, non-GLP), female chinchilla rabbits (3-5/group) were exposed to prothioconazole (purity 98.1-98.8%) at 0, 80, 100, 300, and 480 mg/kg bw/d during GD6-27. Treatment-related deaths occurred in one female at 300 mg/kg bw/d and in two females at 480 mg/kg bw/d. At 480 mg/kg bw/d, total post-implantation loss occurred in one animal and 9/16 foetuses from the remaining two dams in this group were significantly smaller.

In the main developmental toxicity study (OECD TG 414, GLP), female chinchilla rabbits (24/group) were administered prothioconazole (purity 99.5-99.7%) at 0, 10, 30, 80 and 350 mg/kg bw/d during GD6-27. Post-implantation losses were observed in 2, 1, 0, 1 and 3 dams in the control, at 10, 30, 80 and 350 mg/kg bw/d. Other effects were seen in the top dose 350 mg/kg bw/d, one dam died on GD25 following reduced feed consumption and body-weight loss. At the top dose, food consumption was, on average, 31% lower than controls, with a corrected body weight loss of -5% as compared to -1% in controls. No other treatment-related clinical signs were recorded. Three females had abortions. Mean foetal weights were significantly reduced at 350 mg/kg bw/d (10-13% lower than controls). Pre-implantation loss, incidence of dead foetuses and foetal sex ratio were unaffected. There was no clear treatment-related effect on external, visceral and skeletal abnormalities. Incidences of foetuses with abnormalities were similar across all treated groups with no dose-response relationship, which according to the DS did not suggest a treatment-related effect even though the incidence in treated groups was slightly higher than controls. At 350 mg/kg bw/d, the results were highly variable with regard to incidences of incomplete and absent ossification of one or more sternebrae and phalanges of the digits and of unossified 13th rib. In some cases, the incidence was lower than in controls and in others, the incidence was higher. The DS interpreted this variability as being unlikely to be due to prothioconazole exposure. In comparison with the developmental study of prothioconazole in the rat, only one incidence of microphthalmia occurred in this study, in the low dose group and in a foetus with multiple malformations of the head.

Based on the data in rats and rabbits, the DS proposed no classification of prothioconazole for developmental toxicity. The increases of microphthalmia and rudimentary supernumerary ribs occurred in a rat strain with high spontaneous incidences of these changes. Microphthalmia was not increased in another rat strain with negligible spontaneous occurrence of this malformation, even when tested up to doses that were severely toxic to the dams, nor in rabbits at a maternally toxic dose. The DS therefore concluded that the induction of microphthalmia in the first study was a secondary, non-specific exacerbation of a spontaneously occurring malformation in that

strain resulting from maternal toxicity and stress. As supporting evidence, the DS considered the results from an inhalation an oral study with cyfluthrin using the same rat strain. There was an increased frequency of microphthalmia at a dose causing maternal toxicity/stress in the inhalation study at a dose level lower than these that did not cause microphthalmia via the oral route.

The DS analogously concluded that for the increase in rudimentary supernumerary ribs that was observed in the first rat oral study, and marginally in the second rat oral study, was a secondary, non-specific exacerbation of a spontaneously occurring variation in that rat strain. This variation, which is commonly induced together with maternal toxicity is, should not be considered adverse and sufficient for classification.

Comments received during public consultation

Six MSCAs, 1 industry association and 4 individuals (industry associated) commented on reproductive toxicity.

Effects on sexual function and fertility

Two MSCAs requested to consider a possible classification for effects on sexual function and fertility. This request was based on the effects (decreased number of oestrus cycles, implantation sites, litter sizes, corpora lutea; increased oestrus cycle length, mean time to insemination and gestation length) observed in the two-generation rat study at the top dose as these MSCAs considered that the co-occurring maternal toxicity was of only limited severity. The DS responded that they considered the maternal toxicity in the top dose of the range finding-study (500 mg/kg bw/d) and full two-generation study (750 mg/kg bw/d) to be severe. Urine staining and dehydration were observed and they indicated kidney toxicity in line with the observations in the repeated-dose toxicity studies. At a higher dose, 1000 mg/kg bw/d, in the developmental toxicity range-finding study in the same strain of rats, 3/12 dams died of dehydration. Also, reduced food utilisation efficiency leading to decreased body weight gain, and effects on the liver were observed at 750 mg/kg bw/d. The DS considered the observed effects on sexual function and fertility to be slight and likely to be not treatment-related or to be secondary non-specific consequences of maternal toxicity. Two references from the applicant showing impacts of kidney failure on female reproductive parameters such as oestrus cyclicity and ovulation were provided.

One MSCA requested a clarification on the methodology used for normalisation of AGD to body weight. The DS responded that the applicant normalized AGD with the cube root of body weight, as recommended in the literature.

One MSCA, the industry association and 2 individual commenters supported the DS standpoint of no classification for effects on sexual function and fertility.

Effects on development

Two MSCAs asked to consider a possible classification for effects on development, based on the increase in microphthalmia in the first rat study and increased incidence of supernumerary rudimentary 14th rib in both rat studies.

Three MSCAs considered a classification in Cat. 2 warranted. They based their opinion mainly on the increased incidence of microphthalmia outside HCD range in the first rat study, primarily due to the observations in the high dose group but also in the low dose group. The relevance of the study on cyfluthrin was questioned by one MSCA. Since a different pattern of maternal toxicity, caused by hypoxia, was observed, this study was not considered sufficient evidence for a secondary non-specific induction of microphthalmia in this strain of rats after prothioconazole exposure. Possible contributions to the cases of microphthalmia from the metabolites 1,2,4

triazole and prothioconazole-desthio were suggested. The response from the DS was that this particular strain of rat has a high spontaneous incidence of microphthalmia and that the treatment-related increase was an exacerbation of this background incidence, due to severe maternal toxicity at a high dose (1000 mg/kg bw/d). No microphthalmia was observed at a high dose in another rat strain that is sensitive to this malformation, nor in rabbits. With regard to the cyfluthrin study, foetal development was retarded in this study, considered by the DS secondary to maternal toxicity leading to increases in microphthalmia in the same strain of rats in a similar manner as by prothioconazole. The DS considered the reduction of the incidence of microphthalmia in the oxygen-enriched group to reflect the non-specific mode of action for this malformation in this strain of rats. The DS discarded any relevant contributions from 1,2,4 triazole and prothioconazole-desthio to the cases of microphthalmia. These metabolites were formed in rats at a rate of ~2% and ~0.5%, respectively, and additional data from the applicant showed that these metabolites did not cause microphthalmia. The DS also pointed out that prothioconazole was not a triazole (based on information submitted during the public consultation, see below) and that the developmental toxicity of prothioconazole should not be compared with triazole chemicals.

Two individual commenters considered no classification or Cat 2. possible.

One MSCA, the industry association and 1 individual commenter supported no classification for effects on development. Their arguments were primarily based on that microphthalmia was observed in conjunction with maternal toxicity in a strain of rats with a high spontaneous rate of this malformation, which furthermore was not seen in any other study. Rudimentary supernumerary ribs occurred together with maternal toxicity and was considered a variation and not a malformation relevant for classification.

Overall, the DS acknowledged that the findings in two developmental toxicity studies in rats could support either no classification or classification in category 2. The main considerations were the uncertainty of a direct effect of prothioconazole on the occurrence of microphthalmia and the nature and reversibility of the supernumerary ribs, both effects occurring in association with maternal toxicity. However, based on a weight-of-evidence, the DS concluded that the criteria for classification in category 2 were not met and thus proposed no classification of prothioconazole for adverse effects on development.

Assessment and comparison with the classification criteria

Sexual function and fertility

For the evaluation of effects on sexual function and fertility, two reliable GLP-compliant rat studies were presented, a 1-generation dose range-finding study and an OECD TG 416 2-generation study. Based on only minor treatment-related effects in the range-finding study at 500 mg/kg bw/d, a top dose of 750 mg/kg bw/d was chosen for the 2-generation study.

In the 2-generation study, mating, fertility and gestation indices as well as birth, viability and lactation indices were unaffected at all dose-levels in both generations. Effects seen at the top dose consisted of increased mean time to insemination (F1 only) and gestation length, decreased number of oestrus cycles with concurrent increase in cycle length, decreased number of corpora lutea, implantation sites and litter sizes (Table 13). In addition, increased time to preputial separation and increased AGD at birth was reported.

Table 13. *Relevant reproductive parameters of F0 and F1 animals (RAR Table B.6.6.4, modified by RAC).*

Parameter	prothioconazole (mg/kg bw/d)				Historical control data[a]
	0	10	100	750	
F0-generation					
Mean time to insemination (days)	2.6	3.4	2.5	2.9	1.2-3.5
Mean duration of gestation (days)	21.9	21.9	22.1	22.3	21.6-22.1
Mean no. oestrous cycles/14 days	3.4	3.2	3.2	2.7*	-
Mean oestrous cycle duration (days)	4.3	4.2	4.4	5.1*	-
Pre-antral follicles	126.8	-	-	99.4	-
Antral follicles	95.1	-	-	100.1	-
Corpora lutea	62.4	-	-	36.1*	-
Mean no. implants	11.8	11.6	12.2	10.8	9.6-13.3
Mean litter size	10.8	11.1	11.4	10.0	9.4-11.8
F1-generation					
Mean time to insemination (days)	2.4	3.0	3.0	3.8	2.2-3.4
Mean duration of gestation (days)	22.0	22.0	22.2	22.4	21.8-22.2
Mean no. oestrous cycles/14 days	3.6	3.4	3.5	3.1*	-
Mean oestrous cycle duration (days)	4.4	4.5	4.4	4.7	-
Pre-antral follicles	55.2	76.2	70.5	71.8*	35.9-81.6[a]
Antral follicles	42.5	52.9	54.9	54.0	-
Corpora lutea	28.5	22.2	33.6	22.6	-
Mean no. implants	10.7	11.0	11.1	9.3	10.7-11.5
Mean litter size	10.2	10.5	9.7	8.2	9.9-10.8

* $p < 0.05$

In the F0-generation, oestrus cycles were prolonged and a significantly lower number of corpora lutea was measured compared to controls. A concurrent lower mean number of implants and litter size were shown in the high dose group as compared to controls, however within HCD range and without a clear dose-response. Also in the F1-generation, increased oestrus cycle duration was reported as well as a lower number of corpora lutea, however with no dose-response. A lower mean number of implants and litter size was seen in the F1 high dose group as compared to controls, outside HCD range. Also in the mid dose group, a lower mean litter size was shown, outside HCD range. There were no single dams (outliers) responsible for the lower mean litter sizes in the mid and high dose groups.

Although some maternal toxicity was observed in the F0- and F1-generations, RAC does not consider it excessive. At the top dose 750 mg/kg bw/d, in the F0-generation, during pre-mating, decreased body weight gains were -5% in males and -1% in females, and during gestation decreased body weight gains (uncorrected for uterus weight) were -9%/-13% in the F0/F1 generations compared to controls. Cortical nephrosis was observed in 13% and 20% of the F0- (minimal) and F1-generation females (minimal-slight), respectively. With regard to clinical symptoms, urine staining was recorded in the F0- and F1-generations, indicative of renal damage. However, there was no correlation between the individuals showing urine staining and those diagnosed with cortical nephrosis. Dehydration was recorded in a few individuals (males) in the F0-generation (not examined in females) and in 3% of the individuals in the F1-generation (one male and one female) (Table 14).

Table 14. *Summary of clinical signs in F0- and F1-generation parental animals (%) (RAR Table B.6.6.1)*

prothioconazole (mg/kg bw/d)		Males				Females			
		0	**10**	**100**	**750**	**0**	**10**	**100**	**750**
P-Generation									
Salivation prior to dosing	Pre-Mating	0	0	0	**13**	0	0	0	**17**
Urine stain		**3**	0	0	**13**	0	0	0	**57****
Dehydration		0	0	0	**7**	-	-	-	-
Urine stain	Gestation	-	-	-	-	0	0	0	**25***
F1-Generation									
Salivation prior to dosing	Pre-Mating	-	-	-	-	0	0	0	**10**
Urine stain		0	0	0	**13**	0	0	0	**13**
Dehydration		0	0	0	**3**	0	0	0	**3**
Urine stain	Gestation	-	-	-	-	0	0	0	**4**

* Significantly different from control, p ≤ 0.05, ** Significantly different from control, p ≤ 0.01, - Not recorded/observed

The DS provided references in the public consultation linking renal injury to effects on female reproductive parameters. However, the effects observed in those studies were based on severe uremia (recognized as elevated serum urea levels). No such clinical chemistry parameters were measured in the reproductive toxicity studies, but the repeated dose toxicity studies on prothioconazole showed equivocal data on serum urea levels and the changes were of much lower magnitude than those in the provided references.

Thus, RAC concludes that possible classification of prothioconazole is a matter of whether the effects observed in the 2-generation study are sufficient for classification or not, and that the effects observed should not to be discarded due to maternal toxicity.

The effects on preputial separation in the F1-generation and the data obtained from subsequent AGD measurements in F2-pups were also discussed. In the top dose of F1-pups, a significantly delayed preputial separation, outside the range of HCD, was observed. This developmental delay was claimed to be a result of retarded growth during lactation, due to general toxicity. In support of this hypothesis, the applicant provided an analysis of the data with a comparison of individual pup body-weights against the respective days of preputial separation, showing that on each individual day of preputial separation the body-weights of the pups had reached a similar body-weight to that of the controls on the day of their preputial separation. RAC agrees with this conclusion, that the effect was likely secondary to the reduced body-weight gains of the pups at the top dose. In response to the preputial separation observations in the F1-generation, the AGD at birth was measured in the F2-generation. The AGD was slightly, but statistically significantly, larger in both sexes at 750 mg/kg bw/d and in males at 100 mg/kg bw/d. The effect was within the HCD range in all males and was claimed to be due to the higher birth weight of these pups because of longer duration of gestation in F1 dams (0.4 days). When the data for the AGD was normalised for body weight, there were no differences between the high dose and the control groups in either males or females (graph presented in the RCOM document). RAC agrees with the DS that this slight increase in AGD is likely due to increased body weight and not a specific effect of prothioconazole on development.

The effects by prothioconazole in the 2-generation study are by RAC not sufficient for classification for effects on sexual function and fertility. Mating, fertility and gestation indices were unaffected at all dose-levels in both generations as well as parameters of pup viability (birth index, live-birth index, viability index, lactation index). This was supported by similar outcomes in the rat developmental toxicity studies. There were slight changes in oestrus cyclicity, mean time to insemination and gestation time in conjunction with some maternal toxicity that could contribute to these effects. There was a decreased number of corpora lutea in the F0-generation with a possible effect on the number of implants and litter size at the high dose, however within HCD range. This effect was not consistently observed in the F1-generation. The dose, 750 mg/kg bw/d, at which the effects were observed can also be regarded as high (although not excessive).

In a developmental toxicity study in the same strain of rat, mortality occurred at 1000 mg/kg bw/d. Altogether, RAC concludes that prothioconazole is not a specific toxicant to sexual function and fertility, and that the effects are considered incidental, with a possible exacerbation by maternal toxicity, but does not warrant classification.

Development

For the evaluation of the developmental toxicity of prothioconazole, two reliable oral developmental toxicity studies in rats and one in rabbits were available, with associated range-finding studies. One dermal developmental toxicity study in rats was also available, showing no effects.

The first range-finding study showed that prothioconazole was relatively well tolerated in Wistar rats (sub-strain not specified) up to the dose 1000 mg/kg bw/d. No adverse clinical signs were observed and body weight gain was only slightly reduced initially in the beginning of the treatment. As for other repeated-dose studies, increased water consumption and urination occurred, likely due to renal toxicity.

In the first main developmental toxicity study in Hsd Cpb:WU Wistar rats, maternal toxicity was characterized by increased water consumption and urination. Food consumption was significantly reduced in the top dose 1000 mg/kg bw/d during GD6-11, leading to transient body weight loss in the dams on GD6-8. Overall, corrected body weight gain was 31% lower during gestation in the high dose group compared to the control. Increased incidences of microphthalmia compared to control were observed in all dose groups (Table 7), however not dose-related in the low and mid dose groups showing the highest incidence in the low dose group being slightly outside the HCD range on an individual level, but not on a litter basis. The high dose group showed increased foetal and litter incidences of microphthalmia being significantly outside the HCD range, including two cases of bilateral microphthalmia. RAC notes that this strain of Wistar rats has a spontaneous background incidence of this particular malformation, with a high inter-variability between studies (Table 8). During the years 1991-1996, 15/24 studies (including the prothioconazole study) with this strain of rats displayed cases of microphthalmia in the control group. The litter incidences of microphthalmia at 80 and 500 mg/kg bw/d were above the 5-year mean incidence, raising concern among some RAC-members, but below the incidences of several historical control studies conducted one year before/after the prothioconazole study. The foetal incidence at 500 mg/kg bw/d was above the 5-year mean incidence, but lower than the control incidence in several studies, one of which was conducted the same year as the prothioconazole study. The findings of foetal incidences at 80 and 500 mg/kg bw/d above the 5 year-average raised concern among some RAC-members. However, altogether RAC agrees with the DS that the observed cases of microphthalmia in the 80 and 500 mg/kg bw/d dose groups should be considered incidental and not related to treatment, but that the cases at 1000 mg/kg bw/d should be attributed to treatment with prothioconazole. The question is whether the cases of microphthalmia in this dose group is due to a direct effect of prothioconazole or secondary to maternal toxicity. The applicant and the DS grouped the dams giving birth to pups with and without microphthalmia, comparing maternal and foetal body weight (Table 9). The grouping showed that dams giving birth to pups with microphthalmia exhibited a higher body weight loss on GD6-8 than dams giving birth to pups without microphthalmia, and also had an overall lower corrected body weight gain during gestation and gave birth to smaller pups. RAC considers this information to support the assumption that maternal toxicity had an influence on the incidence of microphthalmia in the high dose group, although it is not clear to RAC as to how the maternal toxicity would exacerbate this spontaneously occurring malformation in this strain of rats.

To explore the possible influence of maternal toxicity on the increased incidence of microphthalmia in this strain of rats, a supplementary study was performed where cyfluthrin was administered by inhalation to pregnant rats. In the high dose dams, clinical findings were

apparent and included respiratory disturbances, hypoactivity and decreased food intake/body weight gain (-31%). Lower foetal birth weight (-27%), increased incidence of microphthalmia (5.44% vs 0.41% in the control) and reduced ossification was observed in this group (Table 11). The incidence of microphthalmia was reduced in the high dose group receiving supplementary oxygen (2.9%). No increase in malformations other than microphthalmia was observed. Investigation of the blood showed signs of respiratory alkalosis. Although certain unspecific effects such as decreased maternal body weight gain and decreased foetal birth weight were similar, and correlated with increases in microphthalmia, clinical symptoms were different from dams receiving prothioconazole, and RAC therefore considers this supplementary study on cyfluthrin of limited value for the assessment of prothioconazole.

In the follow-up study using another strain of Wistar rats (Hanover, Crl:WI(HAN)), sensitive to microphthalmia as shown by the teratogen all-trans retinoic acid, no cases of microphthalmia were observed at doses of prothioconazole up to 750 mg/kg bw/d. Maternal toxicity was qualitatively similar with increased water consumption and urination and decreased food intake and body weight gain during gestation, although the magnitude of decreased body weight gain was smaller (corrected body weight gain -13% vs -31% in the first rat developmental toxicity study).

In the rabbit developmental toxicity study, one case of microphthalmia was observed in the low dose group in a foetus with multiple malformations of the head. Further, there were in the 2-generation study no indications of microphthalmia in Hanover, Crl:WI(HAN) Wistar rats at doses up to 750 mg/kg bw/d. RAC acknowledges that the data set with regard to microphthalmia is equivocal.

In addition to microphthalmia, skeletal effects were observed in the offspring in several studies. In the first dose-range finding-study on prothioconazole in rats, a non-dose-related increase in supernumerary rudimentary ribs occurred at 300 and 1000 mg/kg bw/d. Reduced body weight gain occurred in the dams at these doses. In the first full study in Hsd Cpb:WU Wistar rats, a dose-related increase in rudimentary 14th rib was evident at all doses and a decreased ossification occurred in the high dose 1000 mg/kg bw/d, sometimes outside the HCD range. These incidences occurred together with decreased body weight gains in the dams of all dose groups and lower body weights in offspring (outside HCD range at all doses) and with significantly lower body-weight gain and foetal weights in the high dose group. RAC notes that the control incidence for rudimentary 14th ribs was very low, lowest of the 52 studies (out of totally 53 studies) showing this variation in the control group. In the follow up study in Hanover, Crl:WI(HAN) Wistar rats, an increase in rudimentary supernumerary ribs occurred in the top dose 750 mg/kg bw/d, slightly outside HCD range, in conjunction with decreased maternal body weight gain and reduced foetal weight.

In the rabbit developmental toxicity study, effects on ossification were observed, most notable in the high dose group 350 mg/kg bw/d, however the incidence was sometimes higher and sometimes lower than that of the controls, not suggesting a clear effect of treatment. Significant maternal toxicity and reduced foetal weights were evident in the high dose group.

Rudimentary supernumerary ribs are generally regarded as a variation, not a malformation, and to be of low toxicological and biological concern (as opposed to e.g. fully developed supernumerary ribs). This variation is recognised to be induced at maternally toxic doses, as was the case in the present studies, and normally resolves postnatally and does not adversely affect survival or health. Incomplete ossification is commonly viewed as an indication of retarded foetal development and is consistent here with decreased foetal weights. RAC considers the types of variations of limited relevance for classification and recognises that these variations occurred in the presence of some maternal toxicity.

Similarly, the incidence of dilated renal pelvis, commonly considered a developmental delay, occurred in rats at 1000 mg/kg bw/d and is by RAC considered secondary to the slightly retarded growth in these pups.

In the main developmental toxicity study in rabbits, total post-implantation losses were observed in 2, 1, 0, 1 and 3 dams in the control, 10, 30, 80, 350 mg/kg bw/d respectively. Reduced food consumption, body weight gain, one death, abortions and reduced foetal weights occurred only in the high dose group. Together with the treatment-related mortalities that occurred in the dams at 480 and 300 mg/kg bw/d in the dose-range finding study, 1/3 and 2/5 respectively, RAC considers the developmental effects at this dose to be due to maternal toxicity and not sufficient for classification.

In the public consultation, the question whether two identified metabolites, 1,2,4 triazole and prothioconazole-desthio, could contribute to the findings in the developmental toxicity studies was raised. 1,2,4-triazole was identified in urine of rats at ~2% and ~1% after single prothioconazole dosing of 2 and 150 mg/kg bw. The data provided in the public consultation point towards that 1,2,4-triazole does not cause microphthalmia. Together with the low systemic concentration of this substance, RAC agrees with the DS that 1,2,4-triazole likely has no influence on the microphthalmia observed following prothioconazole exposure. Similarly, prothioconazole-desthio, which is a triazole, was found at small amounts in rats following prothioconazole dosing (urine: 0.07%; bile: 0.45%). Prothioconazole-desthio, which has been extensively studied, caused in Wistar Hanover rats (Crl:WI(HAN)) the "classical" triazole pattern of effects including post-implantation loss and cleft palate, but no cases of microphthalmia. Based on the different pattern of effects, together with the low rate of formation of this metabolite, RAC agrees with the DS that prothioconazole-desthio likely has no influence on the effects observed following prothioconazole exposure.

Based on the overall weight-of evidence, RAC is of the opinion that the criteria for classification for adverse effects on development are not met, based on the following:

- The increased incidences of microphthalmia outside HCD range occurred at the limit dose, 1000 mg/kg bw/d, in the Hsd Cpb:WU sub-strain of Wistar rats that has a high background incidence and variability of this particular malformation. The increased incidence in the low and mid dose groups were not dose-related, with the highest incidence in the low dose group 80 mg/kg bw/d, being slightly outside the HCD range on an individual level, but not on a litter basis. These cases are therefore regarded as incidental. The increase of microphthalmia in both foetuses and litters outside HCD range at the top dose are considered related to the treatment. However, these cases occurred together with maternal toxicity. When dams/foetuses were grouped based on maternal toxicity (decreased body weight gain and reduced foetal weight), a clear association was seen between the dams exhibiting more pronounced maternal toxicity and giving birth to smaller pups, with pup/litter incidences of microphthalmia.
- No microphthalmia was seen in the study on prothioconazole carried out in the other strain of rats, Wistar Hanover rats (Crl:WI(HAN), sensitive to this malformation, up to maternally toxic doses. Neither was any microphthalmia detected in the rabbit developmental toxicity study up to maternally toxic doses nor in the rat reproductive toxicity study in Hanover rats (Crl:WI(HAN) at doses up to 750 mg/kg bw/d.
- Increased incidence of supernumerary rudimentary ribs, decreased ossification and dilated renal pelvis, were all associated with delayed foetal development and are not sufficient to warrant classification.

Effects via lactation

In the 2-generation reproductive toxicity study in rats, there were no observed effects on lactation or viability in any of the generations. At the high dose, 750 mg/kg bw/d, pup weight-gain was reduced during lactation (up to 27% and 18%) in F1 and F2 pups with a decreased mean F1 pup weight from day 4 on (10-17%) F2 pup weight from day 7 on (6-12%) compared to controls. Parental toxicity at this dose included lower body weight gains, reduced efficiency of food utilization as well as disruption of normal kidney function and water/electrolyte homeostasis (urine stains, dehydration). Dehydration occurred at this dose-level in rats in several repeated-dose studies and it is possible that also the lactating dams suffered from dehydration, which could affect milk production and weight gain of their pups. The absence of developmental effects at lower doses would support this finding being a non-specific, secondary consequence of the maternal toxicity, not warranting classification.

In summary, RAC agrees with the DS that **no classification of prothioconazole for toxicity to reproduction is warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1, M=10 based on a 72-h estimated mean measured E_rC_{50} value of 0.03278 mg/L for the marine diatom *Skeletonema costatum*, and as Aquatic Chronic 1, M=1 based on lack of rapid degradation and a 72-h estimated mean measured E_rC_{10} value of 0.01427 mg/L for the same species.

Degradation

A hydrolysis study according to EEC method C7 (1992), SETAC (1995), US EPA Guidelines 161-1 (1982) and in compliance with GLP was run at pH 4, 7 and 9 in the dark at 50°C in aqueous buffered solutions. Prothioconazole was stable at pH 7 and 9 where 99.9% and 98.9% (respective mean values) was still present as prothioconazole after 7 days and no degradation products were formed. There was a small amount of hydrolytic degradation seen after 7 days at pH 4. There was 93.3% prothioconazole remaining and formation of JAU 6476-desthio at 5.3%, other degradants accounted for 4.2%. There was less than 10% degradation of the active substance over the course of the 7 day study at all pH values tested. Hydrolytic half-lives of prothioconazole at 50°C were greater than 1 year (pH 7 and 9) and 120 days (pH 4). Therefore, prothioconazole is considered stable to hydrolysis and hydrolytic breakdown is not expected to contribute to its degradation in the environment.

A separate study performed according to EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate, Section 161-1 (1982) and in compliance with GLP demonstrated that metabolite JAU 6476-desthio was hydrolytically stable at pH 5, 7 and 9 at 25°C with less than 6% degradation after 30 days. A degradation DT_{50} value was greater than 1 year at all pH values tested.

Aqueous photolytic degradation was studied according to EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate, Section 161-1 (1982). The study, in compliance with GLP, was carried out in a pH 7 buffer solution at 25°C using a xenon light with a 290 nm

filter and continuous exposure for 18 days, equivalent to 100.7 days in Athens (Greece). The major degradation products detected were JAU 6476-desthio (maximum 55.7% AR after 11 days), JAU 6476-thiazocine (maximum 14.1% AR after 5 days) and 1,2,4-triazole (maximum 11.9% AR after 18 days). There was some evidence of a decline of JAU 6476-desthio and JAU 6476-thiazocine by the end of the study, but 1,2,4-triazole was still increasing. The mean experimental half-lives were 2.1 days for prothioconazole and 54.8 days for JAU 6476-desthio, equivalent to 11.5 and 307 environmental days respectively (summer sunlight conditions in Athens, Greece). According to ECETOC method (1981, 1984) a mean quantum yields Φ of 0.0638 (pH 4) and 0.0047 (pH 9) for prothioconazole and Φ of 0.00449 (in high purity water) for JAU 6476-desthio were calculated.

No ready biodegradability tests were available.

A water/sediment study carried out according to BBA Guideline Part IV, 5-1 (1990) and SETAC Guidelines (1995) and in compliance with GLP, was conducted using two natural systems (Hönniger Weiher (HW) and Anglerweiher (AW)) at 20°C for 121 days. Total recovery of radioactivity was 91.8% to 101.5% applied radioactivity (AR) for HW and 93.7% to 104.2% AR for AW. The amount of un-extracted residues increased during the course of the study and reached a maximum in the sediment of 52.5% AR for HW and 31.3% AR for AW. A maximum of 14.7% AR was recovered as CO_2 for HW and 29.0% for AW. Prothioconazole dissipated rapidly from the water layer in the two different water/sediment systems and was ≤ 2.0% AR by day 14. Partitioning into the sediment occurred rapidly, reaching a maximum of 18.3% AR (HW) and 23.4% AR (AW) one day after application before decreasing to < 10% AR by the end of the study. The metabolite JAU 6476-desthio was rapidly formed, appearing at maximum in the water phase of 13.9% AR (HW) and 32.3% AR (AW) by or before 7 days. Maximum amounts in the sediment were 21.9% AR (HW, day 59) and 26.9% AR (AW, day 14). Four other degradants were present in either the water or sediment at greater than 5% AR. JAU 6476-S-methyl and JAU 6476-triazolinone are formed directly from prothioconazole, while JAU 6476-triazolylketone, and 1,2,4-triazole are formed sequentially from breakdown of JAU 6476-desthio. Only 1,2,4-triazole was greater than 10% AR, reaching a maximum of 37.2% AR (AW). Prothioconazole was dissipated rapidly from the water phase and the geometric mean degradation DT_{50} for the whole system was less than 2 days (adjusted to 12°C). The dissipation DT_{50} value for JAU 6476-desthio from the water column was 18.2 days and the whole system degradation value was 113 days (adjusted to 12°C).

An aerobic mineralisation study (OECD TG 309, GLP) was performed for 60 days at 19.3°C using two nominal test concentrations (10 µg/L and 100 µg/L). Prothioconazole decreased to 54.1% AR (low concentration) and 73.8% AR (high concentration) by the end of the study. The metabolite JAU 6476-desthio was formed, reaching a maximum of 41.9% AR (low concentration) and 29.0% AR (high concentration). No other single component was more than 1.4% AR and carbon dioxide was always ≤ 0.5% AR. The degradation DT_{50} for prothioconazole was 160 days (low concentration) and greater than 1000 days (high concentration).

Overall, although prothioconazole degrades quickly in the whole system of the water/sediment study, the available abiotic and biotic degradation information does not indicate that prothioconazole is ultimately degraded (> 70%) within 28 days (equivalent to a half-life < 16 days) or transformed to entirely non-classifiable degradants. Furthermore, the main degradation product JAU 6476-desthio is hazardous to the aquatic environment. Consequently, the DS considered prothioconazole as not rapidly degradable for the purposes of environmental classification under the CLP criteria.

Bioaccumulation

Prothioconazole has an estimated log P_{OW} of 2.0 at pH 7 and the measured log P_{ow} is in the range 0.2 to 3.4 at 25 °C, depending on pH (OECD TG 117, EC A.8).

A bioconcentration study (OECD TG 305, GLP) is available for prothioconazole. Bluegill sunfish (*Lepomis macrochirus*) were exposed to mean water concentrations (5 and 50 µg/L) of the radiolabelled prothioconazole for 28 days in a flow-through system, followed by 14-day depuration period in clean water. In a second test, bluegill sunfish were exposed to a single concentration (50 µg/L) of radiolabelled prothioconazole for 7 and 14 days to investigate biotransformation of prothioconazole. Prothioconazole accumulates rapidly in bluegill sunfish with a total residue BCF of 43.9 to 57.8 L/kg for whole fish. When exposure ceases, the residues are depurated with a half-life of 0.47 to 0.80 days. After 14 days in clean water 91% (5 µg/L) and 95% (50 µg/L), respectively, of the mean plateau radioactivity were depurated from whole fish. The steady-state BCF (lipid-normalised) was 18.8 L/kg.

In the CLH report, the information on bioaccumulation for the metabolite JAU 6476-desthio was available. The measured mean log P_{ow} of JAU 6476-desthio was 3.04 at 22ºC in unbuffered water (OECD TG 117, EC A.8).

In the bioconcentration study (OECD TG 305, GLP), bluegill sunfish (*Lepomis macrochirus*) were exposed to radiolabelled JAU 6476-desthio at a concentrations of 10 µg/L and 100 µg /L for 28 days in a flow-through test system, followed by 14-day depuration period in clean water. JAU 6476-desthio accumulates rapidly in bluegill sunfish with a total residue kinetic BCF of 71.6 L/kg to 94.3 L/kg for whole fish. The steady-state-BCF (lipid-normalized) was 45 L/kg. When exposure ceases, the residues are depurated with a half-life of 0.39 to 0.47 days. After 14 days in clean water, 96% (10 µg/L) and 99% (100 µg/L), respectively, of the mean plateau radioactivity were depurated from whole fish.

Based on available data on bioaccumulation, the DS concluded that prothioconazole and the major aquatic degradant JAU 6476-desthio do not meet the CLP criteria to be considered as a bioaccumulative substance.

Aquatic toxicity

The ecotoxicological tests results for prothioconazole from the available acute and chronic studies for all three trophic levels are summarised in the following table (the key hazard endpoints and toxicity values used in hazard classification are highlighted in bold). All the studies presented in the table below were considered reliable and relevant by the DS.

Table. *Summary of relevant information on aquatic toxicity of prothioconazole*

Method/Exposure	Test organism	Endpoint	Toxicity values in mg/L	Reference
Short-term toxicity				
OECD TG 203 Static, GLP	*Oncorhynchus mykiss*	96-h LC_{50}	1.83 (mm)	Anonymous, 1999g
OECD TG 203 Static, GLP	*Lepomis macrochirus*	96-h LC_{50}	4.59 (mm)	Anonymous, 1999h
OECD TG 203 Static, GLP	*Cyprinus carpio*	96-h LC_{50}	6.91 (mm)	Anonymous, 2000d
OECD TG 203 Static-renewal, GLP	*Cyprinodon variegatus*	96-h LC_{50}	>10.3 mm	Anonymous, 2004c
OECD TG 202	*Daphnia magna*	48-h EC_{50}	1.3 (nom)**	Heimbach, 1999c

Method/Exposure	Test organism	Endpoint	Toxicity values in mg/L	Reference
Short-term toxicity				
Static, GLP				
OPPTS 850.1035 Flow-through, GLP	*Americamysis bahia*	96-h EC_{50}	2.4 (mm)	Drottar *et al*., 2002a
OPPTS 850.1025 Flow-through, GLP	*Crassostrea virginica*	96-h EC_{50}	2.9 (mm)	Drottar *et al*., 2001
OECD TG 201 Static, GLP	*Pseudokirchneriella subcapitata*	72-h E_rC_{50}	2.18 (im)**	Dorgerloh, 2000b
USEPA Guideline 123-2 (checked against OECD TG 201) Static, GLP	*Skeletonema costatum*	72-h E_rC_{50}	**0.03278 (mm)***	Kern & De Haan, 2004
OPPTS 850.4400 (checked against OECD TG 221) Static-renewal, GLP	*Lemna gibba*	7-d E_rC_{50}	>0.1776 (mm)	Kern *et al*., 2004b
Long-term toxicity				
OECD TG 210 Flow-through, GLP	*Oncorhynchus mykiss*	97-d NOEC	0.308 (mm)	Anonymous, 2001l
OECD TG 210 Flow-through, GLP	*Oncorhynchus mykiss*	91-d NOEC	0.49 (mm)	Anonymous, 2007b
OECD TG 211 Static-renewal, GLP	*Daphnia magna*	21-d NOEC 21-d EC_{10}	0.56 (nom) 0.61 (nom)***	Hendel and Sommer, 2001
OECD TG 219 Static, spiked water, GLP	*Chironomus riparius*	28-d NOEC	9.14 (nom)	Hendel, 2000a
OECD TG 201 Static, GLP	*Pseudokirchneriella subcapitata*	72-h NOE_rC	0.371 (im)**	Dorgerloh, 2000b
USEPA Guideline 123-2 (checked against OECD TG 201) Static, GLP	*Skeletonema costatum*	72-h E_rC_{10}	**0.01427 (mm)***	Kern and De Haan, 2004

Notes:
mm-mean measured concentration; nom-nominal concentration; im-initial measured concentration;

*-72-h endpoint provisional as was estimated by the RMS using 96-h measured concentrations and the ratio between 96-h nominal and mean measured concentration endpoints. RAC noted that in the CLH report and in the DS's response to the public consultation comments (RCOM document), there is a reference that "*the RMS has stated that the Applicant will be requested to calculate these (i.e. 72h) endpoints on mean measured concentrations*", therefore RAC asked EFSA if these additional recalculations for algae *S. costatum* have been generated by the Applicant and were made available to them. In the response, EFSA explained that no request to recalculate the endpoint for this species was finally sent to the Applicant, therefore, no additional calculations were performed by the Applicant.

**-Endpoints based on initial measured or nominal concentrations have been confirmed to be acceptable by the RMS.

*** - Result from the recalculation of the endpoint based on nominal concentrations. The result was not included in the CLH report (explanation provided in section Additional key elements).

Acute toxicity

For fish, four toxicity studies were available. *Oncorhynchus mykiss* was the most sensitive fish species tested in the acute studies, with a 96-h LC_{50} of 1.83 mg/L.

Three toxicity studies were available in case of aquatic invertebrates. *Daphnia magna* was the most sensitive species tested in the acute studies, with a 48-h EC_{50} of 1.3 mg/L.

Three acute toxicity studies were available for algae and aquatic plants. *Skeletonema costatum* was the most sensitive species with a 72-h E_rC_{50} of 0.03278 mg/L.

From the available aquatic toxicity data, algae are the most acutely sensitive trophic group, therefore the acute aquatic classification proposed by the DS was based on the marine diatom *Skeletonema costatum* (72-h E_rC_{50} = 0.03278 mg/L). The DS proposed **Aquatic Acute 1**, with an **M-factor = 10** ($0.01 < L(E)C_{50} \leq 0.1$ mg/L).

Chronic toxicity

For fish, two studies were available. *Oncorhynchus mykiss* was the most sensitive fish species tested in the chronic studies, with a 97-d NOEC of 0.308 mg/L.

Long-term toxicity to invertebrates was assessed based on two available studies that were carried out with *Daphnia magna* and *Chironomus riparius*. The DS concluded that *Daphnia magna* was the most sensitive species tested in the chronic studies, with a 21-d NOEC of 0.56 mg/L.

Two chronic toxicity studies were available for algae. *Skeletonema costatum* was the most sensitive species tested in the chronic studies, with a 72-h E_rC_{10} of 0.01427 mg/L.

The results of long-term aquatic toxicity studies indicate that algae are the most sensitive taxon therefore the chronic aquatic classification proposed by the DS was based on the marine diatom *Skeletonema costatum* toxicity study (72-h E_rC_{10} = 0.01427 mg/L). The DS proposed **Aquatic Chronic 1**, with an **M-factor = 1** ($0.01 < EC_{10} \leq 0.1$ mg/L) along with the understanding that the substance is not rapidly biodegradable.

In the CLH report, the results from aquatic acute and chronic toxicity studies were presented for major degradation product JAU 6476-desthio observed in all available degradation tests (summarised in the following Table). Based on available data the DS concluded that JAU 6476-desthio would be classified as Aquatic Acute 1, M=10 based on a 7-d E_rC_{50} value of 0.0809 mg/L for duckweed *Lemna gibba,* and as Aquatic Chronic 1, M=10 based on lack of rapid degradation and a 97-d NOEC value of 0.00334 mg/L for fish *Oncorhynchus mykiss*. RAC notes that the higher aquatic plant *Lemna gibba* is not the most acutely sensitive trophic group (E_rC_{50} = 0.0809 mg/L). RAC is of the opinion that aquatic invertebrates are the most acutely sensitive trophic group with EC_{50} of 0.060 mg/L for *Americamysis bahia*. However duckweed *Lemna gibba* and saltwater mysids *Americamysis bahia* have acute sensitivity in the same order of magnitude therefore this has no impact on conclusion for acute classification of JAU 6476-desthio.

DS pointed out that the CLH report does not consider the classification of major degradation product JAU 6476-desthio itself but the fact that it would likely be classified for its hazard to aquatic life, is used in determining whether the parent substance prothioconazole should be considered rapidly degradable according to CLP criteria.

Table. *Summary of relevant information on aquatic toxicity of the major aquatic degradant JAU 6476-desthio*

Method/Exposure	Test organism	Endpoint	Toxicity values in mg a.s./L	Reference
Short-term toxicity				
OECD TG 203 Static, GLP	*Oncorhynchus mykiss*	96-h LC_{50}	6.63 (nom)*	Anonymous, 1990
OECD TG 203 Static, GLP	*Leuciscus idus melanotus*	96-h LC_{50}	10.4 (mm)	Anonymous, 1991
OECD EPA-FIFRA 72-1 (checked against OECD TG 203) Static-renewal, GLP	*Pimephales promelas*	96-h LC_{50}	11.4 (mm)	Anonymous, 2003c
OECD TG 202 Static, GLP	*Daphnia magna*	48-h EC_{50}	>10 (nom)*	Heimbach, 1990a
OPPTS 850.1035 Flow-through, GLP	*Americamysis bahia*	96-h LC_{50}	0.060 (mm)	Drottar *et al.*, 2002b
OPPTS 850.1035 Flow-through, GLP	*Americamysis bahia*	96-h LC_{50}	>1.01 (mm)	Blankinship *et al*., 2003
OPPTS 850.1075 Static-renewal, GLP	*Procambarus clarkii*	96-h LC_{50}	>26 (mm)	Sayers, 2004
OPPTS 850.4400 (checked against OECD TG 221) Static-renewal, GLP	*Lemna gibba*	7-d E_rC_{50} 7-d E_rC_{10}	0.0809 (mm) 0.01568 (mm)	Kern *et al.*, 2003
Long-term toxicity				
OECD TG 210 Flow-through, GLP	*Oncorhynchus mykiss*	97-d NOEC	**0.00334 (mm)**	Anonymous, 2002
OECD TG 211 Semi-static, GLP	*Daphnia magna*	21-d NOEC	0.1 (nom)	Dorgerloh and Sommer, 2001c
OPPTS 850.1350 Flow-through, GLP	*Americamysis bahia*	29-d NOEC	0.064 (mm)**	Blankinship *et al.*, 2003
OECD TG 219 Static, spiked water, GLP	*Chironomus riparius*	28-d NOEC 28-d EC_{10}	2.0 (nom) 3.77 (nom)	Hendel, 2000b

Notes:

mm-mean measured concentration; nom-nominal concentration; im-initial measured concentration;

*-Endpoints based on initial measured or nominal concentrations have been confirmed to be acceptable by the RMS.

** - This value is misreported in the CLH report. The correct value is 0.064 mg/L and not 0.0064 mg/L.

Comments received during public consultation

First public consultation

Three Member States (MS) provided public comments, and two agreed with the proposed classification for environmental hazards, one additional MS agreed in the general section of the RCOM. One MS asked for some clarifications regarding the key aquatic chronic toxicity study performed with the marine diatom *Skeletonema costatum* (Kern and DeHaan, 2004) (e.g. validity criteria, use of solvent). The clarification was provided by DS.

Targeted public consultation

During the process of the preparation of the first draft opinion, RAC became aware of additional information generated during the procedure for renewal of the approval of prothioconazole in accordance with Commission implementing regulation (EU) No 844/2012. This additional information was submitted in November 2018 and reviewed by RAC. Additional information provided in November 2018 refers to four new experimental studies performed with two degradation products of prothioconazole. The following studies were submitted: three acute toxicity studies with degradation product JAU 6476-thiazocine carried out on fish *Oncorhynchus mykiss* (OECD TG 203; OCSPP 850.1075, 2016; JMAFF, 2-7-1-1, 2005), invertebrate *Daphnia magna* (OECD TG 202; OCSPP 850.1010, 2016; JMAFF, 2-7-2-1, 2005) and algae *Pseudokirchneriella subcapitata* (OECD TG 201; JMAFF, 2-7-7, 2005) and one acute toxicity study on algae *Desmodesmus subspicatus* (OECD TG 201, OCSPP 850.4500) with degradation product JAU 6476-desthio. Since additional information was submitted, ECHA launched the targeted public consultation (ended on 21.1.2019).

The photolysis degradation product JAU 6476-thiazocine had a lower toxicity to fish *Oncorhynchus mykiss* (96-h LC_{50} > 17.8 mg/L (mean measured)), invertebrate *Daphnia magna* (48-h EC_{50} > 18.6 mg/L (mean measured)) and algae *Pseudokirchneriella subcapitata* (72-h E_rC_{50} > 17.3 mg/L (mean measured)) than the parent compound prothioconazole and, therefore, not considered further for classification of prothioconazole.

One Member State (from the Competent Authority of the DS) submitted a comment during the targeted public consultation. It explained the basis for the aquatic hazard classification proposal and pointed out that new aquatic toxicity studies submitted via the pesticide review for prothioconazole do not affect the original aquatic classification proposal, which is based on prothioconazole and JAU 6476-desthio data.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider prothioconazole as not rapidly degradable. The substance does not undergo rapid abiotic degradation (the hydrolysis DT_{50} is >1 year (pH 7 and 9) and >120 days (pH 4) at 50°C). No ready biodegradation screening test is available. No significant degradation in the aerobic mineralisation study was observed. The results of the water/sediment simulation study showed that prothioconazole dissipates rapidly from the water phase (geomean water dissipation DT_{50} was 0.73 days) and the loss from the whole system was also rapid (the geometric mean degradation DT_{50} was 1.6 days). The main degradation product formed in all available tests was JAU 6476-desthio, which would be classified for environmental hazards as Aquatic Acute 1 (M=10) and Aquatic Chronic 1 (M=10). In addition, the degradant 1,2,4-triazole was formed in water but there was no clear evidence of degradation. Therefore, the substance is considered to be not rapidly degradable for the purposes of environmental classification.

Bioaccumulation

RAC agrees with DS that prothioconazole has a low potential to bioaccumulate in aquatic organisms. The basis for this is that estimated log P_{ow} value of 2.0 and measured log P_{ow} in the range 0.2 to 3.4 is below the CLP Regulation threshold of 4 and the measured whole fish BCF value of 18.0 L/kg is below the CLP Regulation criterion of 500.

Aquatic toxicity

Reliable short-term aquatic toxicity data are available for fish, invertebrates, algae and higher aquatic plants and the lowest estimated mean measured 72-h E_rC_{50} value is 0.03278 mg/L for the marine diatom *Skeletonema costatum*. As this concentration is below the threshold value of 1 mg/L, RAC concludes that a classification as Aquatic Acute 1 (H400) is warranted. As 0.01 < E_rC_{50} ≤ 0.1 mg/L, the acute M-factor is 10.

Reliable long-term aquatic toxicity data are available for all three trophic levels. The lowest chronic effect value corresponds to a test with marine diatom *Skeletonema costatum* with an estimated mean measured 72-h E_rC_{10} of 0.01427 mg/L. As the value is below the threshold value of 0.1 mg/L for not rapidly degradable substances, RAC concludes that a classification as Aquatic Chronic 1 (H410) is justified. As 0.01 < EC_{10} ≤ 0.1 mg/L, the chronic M-factor is 1.

In summary, on the basis of the available data, RAC agrees with the DS that prothioconazole should be classified as: **Aquatic Acute 1 (H400), M-factor = 10** and **Aquatic Chronic 1 (H410), M-factor = 1.**

RAC evaluated the new information provided through the procedure for the renewal of the approval of prothioconazole in accordance with Commission implementing regulation (EU) No 844/2012 but considered that it did not alter the classification of prothioconazole as proposed by DS.

Additional references

References supporting the validity of the modified mouse LLNA

Vohr *et al.*: Detection of photoreactivity demonstrated in a modified local lymph node assay in mice. Photoderm. Photoimm. & Photomed., 10, 57 (1994).

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Homey *et al.*: An integrated Model for the Differentiation of Chemical-Induced Allergic and Irritant Skin Reactions (IMDS). Toxicol. and Appl. Pharmacol., 153, 83-94 (1998).

Vohr, *et al.*: An intra-laboratory validation of IMDS: Discrimination Between (Photo)Allergic and (Photo)Irritant Skin Reactions in Mice. Arch. Toxicol., 73, 501-509 (2000).

ANNEXES:

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

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

Annex 3 Records of the targeted Public Consultation following submission of additional experimental information on aquatic species generated during the procedure for renewal of the approval of prothioconazole in accordance with Commission implementing regulation (EU) No 844/2012