Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 March 2019

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance name: PROTHIOCONAZOLE

EC Number: **Not allocated**

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

Index Number: **Not allocated**

Contact details for dossier submitter: **UK Competent Authority**
Chemicals Regulation Division
Health and Safety Executive
United Kingdom

Version number: 2 **Date: March 2018**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PROTHIOCONAZOLE (ISO); 2-[2-(1-CHLOROCYCLOPROPYL)-3-(2-CHLOROPHENYL)-2-HYDROXYPROPYL]-2,4-DIHYDRO-3H-1,2,4-TRIAZOLE-3-THIONE

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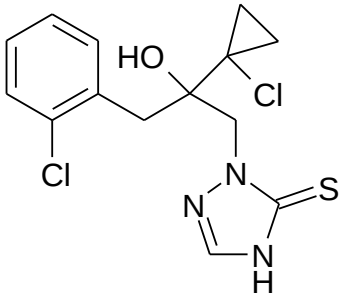
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	IUPAC name: (RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione CAS name: 3*H*-1,2,4-Triazole-3-thione, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-
Other names (usual name, trade name, abbreviation)	Prothioconazole
ISO common name (if available and appropriate)	Prothioconazole
EC number (if available and appropriate)	not allocated
EC name (if available and appropriate)	not allocated
CAS number (if available)	178928-70-6
Other identity code (if available)	CIPAC number: 745
Molecular formula	$C_{14}H_{15}Cl_2N_3OS$
Structural formula	 Racemate (50:50)
SMILES notation (if available)	C1C(Cl)(C(O)(CN3N=CNC3=S)CC2=CC=CC=C2Cl)C1
Molecular weight or molecular weight range	344.26 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable: The active substance is a racemate.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable: The substance is not an UVCB.
Degree of purity (%) (if relevant for the entry in Annex VI)	min. 970 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Prothioconazole	min. 97.0% w/w	No entry in Annex VI	

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Toluene CAS name: benzene, methyl-) [108-88-3]	max. 0.5 %			
Prothioconazole-desthio (CAS name: 1H-1,2,4-Triazole-1-ethanol, α-(1-chlorocyclopropyl)-α-[(2-chlorophenyl)methyl]-, (+/-)) [120983-64-4]	max. 0.05%			

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant	-	-	-	-	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Dossier submitters proposal	n.a.	Prothioconazole	605-841-2	178928-70-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	P273	M = 10 M = 1	n.a.
Resulting Annex VI entry if agreed by RAC and COM		Prothioconazole	605-841-2	178928-70-6	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	P273	M = 10 M = 1	n.a.

n.a.: not applicable

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier; hazard class not applicable	No
Oxidising gases	hazard class not assessed in this dossier; hazard class not applicable	No
Gases under pressure	hazard class not assessed in this dossier; hazard class not applicable	No
Flammable liquids	hazard class not assessed in this dossier; hazard class not applicable	No
Flammable solids	data conclusive but not sufficient for classification	Yes
Self-reactive substances	data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	hazard class not assessed in this dossier; hazard class not applicable	No
Pyrophoric solids	hazard class not assessed in this dossier; hazard class not applicable	No
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier; hazard class not applicable	No
Oxidising liquids	hazard class not assessed in this dossier; hazard class not applicable	No
Oxidising solids	data conclusive but not sufficient for classification	Yes
Organic peroxides	hazard class not assessed in this dossier; hazard class not applicable	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	hazard class not assessed in this dossier; data lacking	No
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	hazard class not assessed in this dossier; data lacking	No
Hazardous to the aquatic environment	harmonised classification proposed	Yes
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Prothioconazole is an active substance in the scope of the Regulation (EC) 1107/2009 (repealing Directive 91/414/EEC). The substance is not currently listed in Annex VI of CLP, and there have been no previous classification and labelling discussions of this substance. The substance is therefore subject to the harmonised classification and labelling process in accordance with Article 36(2) of CLP and no further justification is required.

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.

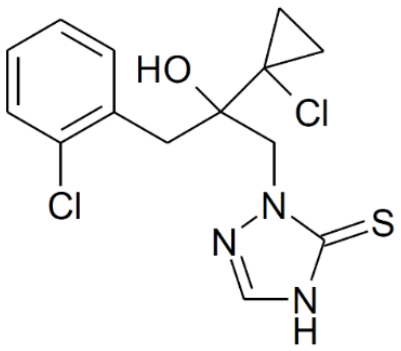


Figure 1. Molecular structure of prothioconazole.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

Prothioconazole is used in foliar and seed treatment to control diseases caused by pathogen fungi from the three classes Ascomycetes, Deuteromycetes and Basidiomycetes.

6 DATA SOURCES

Studies which have been submitted for Annex I renewal under 1107/2009.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101,3 kPa	PAS: White powder TGAS: Light beige powder	Ziemer, F.; Strunk, B.; 2014 Ziemer, F.; 2015	Observed
Melting/freezing point	mp = 140.3 °C	Nau, M.; 2014	Measured
Boiling point	No boiling point at atmospheric pressure	Nau, M.; 2014	Measured
Relative density	PAS: D_4^{20} = 1.38 TGAS: D_4^{20} = 1.39	Ziemer, F.; Strunk, B.; 2014	Measured
Vapour pressure	7.4×10^{-10} Pa at 20 °C 1.8×10^{-9} Pa at 25 °C 1.1×10^{-7} Pa at 50 °C	Dreisch, S.; 2014	Extrapolated
Surface tension	67.4 mN/m at 20 °C	Eyrich, U.; Ziemer, F.; Peschke, C., 2014	Measured
Water solubility	**Buffer** **Solubility** pH 4 2.20 mg/L pH 7 22.5 mg/L pH 9 1.24 g/L	Ziemer. F.; Strunk, B.; 2014	Measured
Partition coefficient n-octanol/water	**Buffer** **log Pow** pH 4 3.4 pH 7 2.0 pH 9 0.2	Ziemer, F.; Strunk, B.; 2014	Measured
Flash point	Not applicable.	-	The substance is a solid.
Flammability	Not highly flammable according to EU A.10	Winkler, S.; 2015	Measured

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Explosive properties	Not explosive in the sense of EC A.14 and OECD 113. Not explosive in the sense of UN RTDG	Winkler, S.; 2015 Dreisch, S.; 2016	Measured
Self-ignition temperature	No spontaneous combustion observed according to Bowes-Cameron-Cage test. No self-ignition temperature was observed up to 403 °C according to method A.16	Heitkamp, 2000 Winkler, S.; 2015	Measured
Oxidising properties	The substance has no oxidizing properties in the sense of EC A.17	Winkler, S.; 2015	Measured
Granulometry	Not available	-	-
Stability in organic solvents and identity of relevant degradation products	Not available	-	-
Dissociation constant	pKa = 6.8	Wiche, A.; Ziemer, F.; 2014	Measured
Viscosity	Not required	-	The substance is a solid.

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Method EC A.14	Not explosive in the sense of EC A.14 and OECD 113		Winkler, S.; 2015
Koenen test Time pressure test Trautzl test	Negative in all three tests		Dreisch, S.; 2016

8.1.1 Short summary and overall relevance of the information provided on explosive properties

The thermal stability pre-test by means of DSC measurement showed an exothermal effect in the temperature range of 215 – 440 °C with a heat of decomposition above 500 J/g (Energy release > -1013 J/g)

The main test according to the guidance EC A.14 showed no explosion in the mechanical sensitivity test (friction and shock) and no explosion in the thermal sensitivity test with 2 mm hole nozzle.

Further tests according to the UN Recommendations on the Transport of Dangerous Goods, showed to be all negative, i.e. the Koenen test showed no explosion when using a 1.0 mm diameter orifice plate; in the Time Pressure test, the test item did not reach a pressure of 2070 kPa; the Trauzl test resulted in an expansion of 1.7 mL per 10 g.

8.1.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

8.1.3 Conclusion on classification and labelling for explosive properties

The substance does not have explosive properties. Data conclusive but not sufficient for classification.

8.2 Flammable gases (including chemically unstable gases)

Table 9: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Hazard class not applicable: The substance is a solid.

8.2.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a solid.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable: The substance is a solid.

8.3 Oxidising gases

Table 10: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Hazard class not applicable: The substance is a solid.

8.3.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a solid.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable: The substance is a solid.

8.4 Gases under pressure

Table 11: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Hazard class not applicable: The substance is a solid.

8.4.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a solid.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable: The substance is a solid.

8.5 Flammable liquids

Table 12: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Hazard class not applicable: The substance is a solid.

8.5.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a solid.

8.5.3 Conclusion on classification and labelling for flammable liquids

Hazard class not applicable: The substance is a solid.

8.6 Flammable solids

Table 13: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Method EC A.10	Not highly flammable according to EU A.10	-	Winkler, S.; 2015

8.6.1 Short summary and overall relevance of the provided information on flammable solids

The substance melted during the standardized pre-test according to guidance EU A.10 and could not be ignited with a flame.

8.6.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

8.6.3 Conclusion on classification and labelling for flammable solids

Not a flammable solid. Data conclusive but not sufficient for classification.

8.7 Self-reactive substances

Table 14: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Method EC A.16	No exothermic effects at temperatures up to 420 °C. The substance does not undergoes spontaneous combustion in the sense of EC A.16		Heitkamp, 2000
Method EC A.16	No self-ignition temperature for the technical substance was observed up to the maximum test temperature of 403 °C.		Winkler, S.; 2015

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

The substance does not undergo spontaneous combustion in the sense of EC guideline A.16.

8.7.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Not a self-reactive substance. Data conclusive but not sufficient for classification.

8.8 Pyrophoric liquids

Table 15: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Hazard class not applicable: The substance is a solid.

8.8.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a solid.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Hazard class not applicable: The substance is a solid.

8.9 Pyrophoric solids

Table 16: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Based on experience in manufacture and handling the substance does not ignite spontaneously on coming into contact with air at normal temperatures. Thus, the study does not need to be conducted according to Regulation (EC) No 1272/2008, Annex I, part 2 (2.10.4.1).

8.9.2 Comparison with the CLP criteria

Hazard class not assessed in this dossier; hazard class not applicable.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

8.10 Hazard class not assessed in this dossier; hazard class not applicable.Self-heating substances

Table 17: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
UN N.4 (Bowes-Cameron-Cage Test)	The substance does not undergo spontaneous combustion in 1 L Bowes-Cameron-Cage test	-	Heitkamp, 2000
EC A.16	The substance shows no self-ignition temperature up to 403 °C.	-	Winkler, S.; 2015
EC A.1 and OECD 102	mp = 140.3 °C	-	Nau, M.; 2014

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

The self-heating properties have been studied according to the standard procedure described in the UN Manual of Tests and Criteria, test N.4. The test shows the substance does not undergo spontaneous combustion after 24 h at 140 °C.

A standardized determination of the self-ignition temperature as described in the guidance EC A.16 shows no self-ignition temperature up to 403 °C.

Additionally, the melting point of the substance is 140.3 °C. Substances with a melting point below 160 °C should not be considered for classification in the self-heating hazard class since the melting point is an endothermic process.

8.10.2 Comparison with the CLP criteria

The substance does not meet the criteria for classification for this hazard class.

8.10.3 Conclusion on classification and labelling for self-heating substances

Not a self-heating substance or mixture. Data conclusive but not sufficient for classification.

8.11 Substances which in contact with water emit flammable gases

Table 18: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class.

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Based on the chemical structure of the substance and the experience in manufacture and handling the substance does not react with water. Thus, a study does not need to be conducted according to Regulation (EC) No 1272/2008, Annex I, part 2 (2.12.4.1).

8.11.2 Comparison with the CLP criteria

8.11.3 Hazard class not assessed in this dossier; hazard class not applicable.Conclusion on classification and labelling for substances which in contact with water emit flammable gases

8.12 Hazard class not assessed in this dossier; hazard class not applicable.Oxidising liquids

Table 19: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class.

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Hazard class not applicable: The substance is a solid.

8.12.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is a solid.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Hazard class not applicable: The substance is a solid.

8.13 Oxidising solids

Table 20: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC A.17	The substance has no oxidizing properties in the sense of EC A.17	-	Winkler, S.; 2015

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

In the standardized main test as described in the guidance EC A.17, the substance burned slower compared to the reference mixture and thus it is concluded that the test item has no oxidizing properties according the guidances EC A.17.

8.13.2 Comparison with the CLP criteria

The classification procedure for oxidising substances does not need to be applied for organic compounds if the compound contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.

8.13.3 Conclusion on classification and labelling for oxidising solids

Data conclusive but not sufficient for classification.

8.14 Organic peroxides

Table 21: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
-	-	-	-

No studies necessary for this hazard class.

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Hazard class not applicable: The substance is not a peroxide.

8.14.2 Comparison with the CLP criteria

Hazard class not applicable: The substance is not a peroxide.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable: The substance is not a peroxide.

8.15 Corrosive to metals

Table 22: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
-	-	-	-

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No test data are available. However, based on the experience in manufacture and handling the substance does not materially damage metallic containers.

8.15.2 Comparison with the CLP criteria

8.15.3 Hazard class not assessed in this dossier.Conclusion on classification and labelling for corrosive to metals

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**.

9 HAZARD CLASS NOT ASSESSED IN THIS DOSSIER.TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 23: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Biokinetic Behaviour and Metabolism (ADME) in the Rat OECD 417 (1984); US-EPA 712–C–98–244, OPPTS 870.7485 (1998) GLP	Rapid and nearly complete absorption; broad distribution, but primarily to liver and kidney; almost complete excretion within 48 hours of oral administration; extensive metabolism to 18 metabolites, with the major metabolic reactions being S-conjugation with glucuronic acid, oxidative hydroxylation of the phenyl moiety, and desulfuration (almost exclusively in the faeces)	[triazole-UL-^{14}C]Prothioconazole: 5 male & 5 female rats at 2 mg/kg bw; 5 male & 5 female rats at 150 mg/kg bw; 8 male bile-duct cannulated rats at 2 mg/kg bw [phenyl-UL-^{14}C]Prothioconazole: 5 male rats at 5 mg/kg bw; 5 male rats at 2 mg/kg bw ($^{14}CO_2$ test); 20 male bile-duct cannulated rats at 2 mg/kg bw; 5 male & 5 female rats repeatedly dosed at 2 mg/kg	Anonymous, 2001a

Method	Results	Remarks	Reference
		bw	
Distribution of the Total Radioactivity in Rats Determined by Quantitative Whole Body Autoradiography (QWBA) OECD 417 (1984); US-EPA 712–C–98–244, OPPTS 870.7485 (1998) GLP	Broad distribution, but primarily to liver and kidney; continuous decrease of the radioactivity concentrations in organs and tissues within the test period of 7 days	[triazole-UL-^{14}C]Prothioconazole: 8 male & 8 female rats at 4 mg/kg bw	Anonymous, 2001b
Metabolic Stability and Profiling in Liver Microsomes from Rats and Humans for Inter-Species Comparison GLP	The metabolic pattern in rat and human liver microsomes were qualitatively very similar and no unique human metabolite was detected.	[phenyl-UL-^{14}C]Prothioconazole: *in vitro* (10 µM) with liver microsomes from male rats and humans in the presence of NADPH cofactor	Anonymous, 2014
In vitro metabolism and detoxification in human and rat hepatocytes	The principal metabolic reactions were identical in both species. S-conjugation with glucuronic acid was the major detoxification route in both species.	[triazole-UL-^{14}C]Prothioconazole: *in vitro* (2.9 µM) with hepatocytes from male rats and humans	Anonymous, 2015

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

In vivo studies

Prothioconazole was almost completely absorbed via the intestinal tract. At least ca. 90% of the administered dose was absorbed at the low dose level (2 mg/kg bw) by male rats, as was calculated from the bile-duct cannulation experiment using the triazole-^{14}C-labelled compound. The rate and extent of absorption of the total radioactivity was essentially independent of sex and labelling position.

The radioactivity administered with prothioconazole was widely distributed over various organs (plasma t_{max} = 0.1 – 0.7 h), with rapid decrease of residues to levels close to or at the limit of quantification after 72 hours for most organs and tissues. The radioactivity was at least partially subject to an enterohepatic circulation, as was concluded from the oscillating plasma concentration curves. The excretion of radioactivity was almost complete by 48 hours after oral administration of triazole- or phenyl-^{14}C-labelled prothioconazole. In almost all tests, between approximately 90 and 100% of the administered dose was excreted with urine, faeces, or bile at the time of sacrifice, i. e. 48 hours or 168 hours after dosing. About 78 - 96% of the administered dose was excreted with the faeces and only ca. 4 – 16% renally. The residues in the body at sacrifice ranged from ca. 0.1 to 1.5% of the administered dose for the animals sacrificed after 168 hours and from 1 to 6% for the animals sacrificed 48 hours following administration. By far the greatest amounts of radioactivity were found in the organs responsible for the absorption, degradation, and excretion, i. e., in the gastrointestinal tract, liver, and kidney. Consequently, less than 0.5% of the administered dose was found in all remaining organs and tissues. There was no evidence of accumulation. In a quantitative whole body autoradiography study in rats, a continuous decrease of the radioactivity concentrations in the organs and tissues by several orders of magnitude was observed within the test period of 7 days; distribution to the blood and bone marrow after a single oral dose was demonstrated.

Prothioconazole was extensively metabolised in the rat. Eighteen metabolites, including the parent compound, were identified in urine, faeces, and bile. The overall most abundant metabolite was prothioconazole-S-glucuronide (ca. 46% of the administered dose in the bile and up to 7.8% in the urine), followed by the

unchanged parent compound (ca. 1 – 22%) and prothioconazole-desthio (ca. 0.4 – 18%). Prothioconazole-desthio was found almost exclusively in the faeces and only to a very minor extent systemically (urine: max. 0.07%, bile: max. 0.45%). The S-glucuronide conjugate has a higher polarity than the parent and is therefore rapidly excreted. Furthermore, this conjugation results in the sulphur being protected against cleavage. Thereby the chemical modification of the triazolinethione moiety to a triazole is prevented, meaning that no relevant amount of the metabolite prothioconazole-desthio can be formed in animals. All metabolites present in the excreta at amounts ≥ 5% and many other metabolites accounting for less than 5% of the administered dose were identified.

In vitro studies

After 0.5 and 1 hours' incubation with liver microsomes from male rats and humans in the presence of NADPH cofactor, the metabolite profile of ^{14}C-prothioconazole was found to be slightly different between rats and humans. In incubations with rat liver microsomes, prothioconazole was metabolised to eleven metabolites, of which three were above 5% of the relative percentage, whereas approx. 50 – 60% of the initial ^{14}C-prothioconazole remained unchanged. In human liver microsomes, ^{14}C-prothioconazole was metabolised to a lower number of metabolites and the amount of unchanged ^{14}C-prothioconazole following an incubation period of one hour was considerably higher (approx. 90%) as compared with rat liver microsomes. This indicated a slower metabolism rate in human liver microsomes. The metabolic pattern in rat and human liver microsomes was qualitatively very similar and no unique human metabolite was detected.

After 2 hours' incubation with hepatocytes from male rats and humans, prothioconazole was extensively metabolised and very low amounts of the test compound remained unchanged (less than 4%). The principal metabolic reactions were identical in both species. Conjugation of the parent compound with glucuronic acid at the sulphur atom of the molecule (prothioconazole-S-glucuronide) was the major detoxification route in both species (maximum 34.5% in human and 55.5% in rat hepatocytes). A further important detoxification route observed in both species was the conjugation of the hydroxymethoxy and hydroxy metabolites of prothioconazole with glucuronic acid. The sum of these glucuronide conjugates were in both *in vitro* systems very similar, i.e. approx. 65%. Prothioconazole-desthio was found only at low amounts (maximum 4.7% in human and 4.0% in rat hepatocytes).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

The acute toxicity of prothioconazole has been investigated via the oral, dermal and inhalation routes of exposure. Additionally, acute skin and eye irritation studies are available. Skin sensitisation has been investigated in a guinea-pig maximisation test and a modified mouse local lymph-node assay.

10.1 Acute toxicity - oral route

The acute oral toxicity of prothioconazole has been investigated in rats.

Table 24: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}
Acute Oral Toxicity (Acute Toxic Class Method) OECD 423 (1996) GLP Anonymous,	Rat, Wistar, Hsd Cpb:WU Males & Females 3/sex/group	Prothioconazole (purity 99.8 %) Vehicle: 2 % aqueous Cremophor EL.	5000 mg/kg bw (Test concentration was found to be 24 % higher than nominal. Actual dose level: 6200 mg/kg bw) Single gavage dosage	> 6200 mg/kg bw

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}
1998a				

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

The acute oral toxicity of prothioconazole has been evaluated in rats, using the acute toxic class method. A single gavage dose of 5,000 mg/kg bw/day (actual test concentration of 6,200 mg/kg bw/day) was administered to groups of 3/sex Wistar rats. 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 signs of gross necropsy were noted. The LD_{50} value for acute oral toxicity was therefore > 6200 mg/kg bw.

10.1.2 Comparison with the CLP criteria

The guidance on the application of the CLP criteria (Regulation (EC) 1272/2008) gives a cut-off LD_{50} value of 2000 mg/kg bw for the classification of acute toxicity via the oral route. Under the conditions of this study the LD_{50} value of prothioconazole for oral toxicity was found to be > 6200 mg/kg bw. Therefore no classification for acute oral toxicity is proposed.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Not classified – Conclusive but not sufficient for classification

10.2 Acute toxicity - dermal route

The acute dermal toxicity of prothioconazole has been investigated in rats.

Table 25: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}
Acute Dermal Toxicity OECD 402 (1987) GLP Anonymous, 1999a	Rat, Wistar, Hsd Cpb:WU Males & Females 5/sex/group	Prothioconazole (purity 98.8%)	2000 mg/kg bw 24 hours, semi-occlusive	> 2000 mg/kg bw

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

The acute dermal toxicity of prothioconazole has been investigated in rats. Groups of 5/sex Wistar rats were administered 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. The treated skin showed signs of reddening (in males and females) and partial encrustation (in females) on days 2-8. Body weight gain during the observation period was minimal in males and absent in females. The applicant attributes this to the age of the females at dosing (15 weeks), where minimal body weight gain would be expected. The LD_{50} value for the acute dermal toxicity of prothioconazole in the rat was > 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

The application on the guidance of the CLP criteria (Regulation (EC) 1272/2008) gives a cut off LD_{50} value of 2000 mg/kg bw for acute dermal toxicity classification. Under the conditions of this study prothioconazole had an LD_{50} of > 2000 mg/kg bw and as such no classification for acute dermal toxicity is proposed.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified – Conclusive but not sufficient for classification

10.3 Acute toxicity - inhalation route

The acute toxicity of prothioconazole via the inhalation route has been investigated in rats.

Table 26: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}
Acute Inhalation Toxicity OECD 403 (1987) GLP Anonymous, 1999b	Rat, Wistar HSD Cpb:WU Males & Females 5/sex/group	Prothioconazole (purity 98.8 %), Dust aerosol MMAD ± GSD: 3.85 ± 2.06 µm	5 mg/L (mean) 4 hours, nose-only	> 5 mg/L (4 hours)

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The acute inhalation toxicity of prothioconazole has been investigated in a nose-only inhalation study in rats. Wistar rats were exposed for four hours to the maximum attainable concentration of 5 mg/L prothioconazole administered as a dust (solid aerosol). 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 had resolved by day 3 of the investigation and were attributable to non-specific responses to dust exposure. There were no treatment-related gross necropsy findings in any animal. The 4-hour LC_{50} value of prothioconazole was > 5 mg/L.

10.3.2 Comparison with the CLP criteria

The guidance of the application of the CLP criteria (Regulation (EC) 1272/2008) gives a 4-hour LC_{50} cut-off value of 5 mg/L to trigger classification for acute inhalation toxicity. Under the conditions of this study the 4-hour LC_{50} was > 5 mg/L, which was the maximum concentration attainable. No classification for acute inhalation toxicity is warranted.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified – Conclusive but not sufficient for classification.

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**.

10.4 Skin corrosion/irritation

The potential of prothioconazole to induce acute skin corrosion or irritation has been investigated in rabbits.

Table 27: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility
Acute Skin Irritation (Patch Test) OECD 404 (1992) GLP Anonymous, 1996a	Rabbit, Himalayan Males 3/group	Prothioconazole (purity 99.8 %)	500 mg (powder, moistened with water prior applying to the skin) 4 hours, semi-occlusive	- None of the three rabbits showed any substance-related lesions at the examination time-points 1, 24, 48 and 72 hours after patch removal. - mean score: 0 (in 3/3 animals)

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The skin irritation potential of prothioconazole was investigated in the rabbit. The moistened test material was applied to the shorn skin of three rabbits. Observation for skin reactions was carried out at 1, 24, 48 and 72 hours. There were no skin reactions observed at any of these time points.

10.4.2 Comparison with the CLP criteria

The guidance on the application of the CLP criteria (EC 1272/2008) requires that mean irritation score are > 2.3 for erythema/eschar in at least 2 out of 3 animals, before classification as a skin irritant is triggered. The scores for erythema/eschar for prothioconazole in this study were 0 for all animals. Prothioconazole was found to be not irritating to the skin of the rabbit under the conditions of this study, therefore no classification for skin corrosion/irritation is proposed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified – Conclusive but not sufficient for classification

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**.

10.5 Serious eye damage/eye irritation

The potential of prothioconazole to cause serious eye damage or irritation has been investigated in rabbits.

Table 28: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility
Acute Eye Irritation OECD 405 (1987) GLP	Rabbit, Himalayan Males 3/group	Prothioconazole (99.8 %)	100 mg	- Eyes were examined and irritation was assessed at 1, 24, 48 and 72 hours after administration (including fluorescein at 24 hours); mean scores were calculated from 24-72 h values - Mean scores: 0 in 3/3 animals

Anonymous, 1996b				(only minimal conjunctival redness (grade 1) was observed in a single animal at 1 hour only)

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The eye irritating potential of prothioconazole was investigated in rabbits. A single dose of 100mg of powdered prothioconazole was administered to one eye each of three Himalayan rabbits. Eye irritation was assessed 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. Under the conditions of this study prothioconazole is not irritating to the eye of the rabbit.

10.5.2 Comparison with the CLP criteria

The guidance on the application of the CLP criteria (EC 1272/2008) lists the following 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 the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses after 21 days.

These criteria were not met at any observation point for any animal in the study. Therefore no classification for serious eye damage/eye irritation is proposed.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified – Conclusive but not sufficient for classification

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**.

10.6 Respiratory sensitisation

No data on respiratory sensitisation available. Prothioconazole was negative in two skin sensitisation studies (see below); therefore, it is unlikely that it would induce respiratory sensitisation.

10.6.1 Conclusion on classification and labelling for respiratory sensitisation

No classification proposed.

10.7 Skin sensitisation

The skin sensitising potential of prothioconazole has been investigated in a guinea-pig maximisation test and a mouse local lymph-node assay.

Table 29: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results
Guinea-pig maximisation test (GPMT) OECD 406 (1992) Deviations: 10 (main) and 5 (control) animals instead of 20 & 10 GLP Anonymous, 1996c	Guinea pig, Hartley (Hsd Poc:DH), males, 10 in test groups & 5 in control groups	Intradermal induction 5% test substance, Freund's complete adjuvant (FCA) 1:1 with sterile physiological saline solution containing 2% cremophor EL Topical induction 25% test substance (48 hrs) Challenge 12 % test substance, Prothioconazole purity 99.8 %	**Negative** No. sensitised animals / total no.: Test substance group: 1/10 Naïve prothioconazole control: 0/5 Minimal skin irritation (grade one on a four point scale) was recorded in a single treated animal at 24 and 48 hours after exposure

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Results
Local Lymph Node Assay (LLNA/IMDS) OECD 429 (2002) GLP Anonymous, 2007a	Mouse, Hsd Win:NMRI, females 6/group	Vehicle: dimethyl-formamide 0 %, 2 %, 10 % and 50 % Epicutaneous application on dorsal part of both ears (25 µL/ear) on 3 consecutive days Prothioconazole (purity 97.2 %)	**Negative** Cell counts and weights of the draining lymph nodes: no increase in stimulation indices in any dose group. Ear swelling and ear weight: no increase in any dose group

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The skin sensitising potential of prothioconazole was investigated in a guinea-pig maximisation test and a modified mouse local lymph-node assay. Prothioconazole was not a skin sensitiser in either of these investigations.

In a standard guinea-pig maximisation test according to Magnusson and Kligman, an intra-dermal induction of 5% was administered to 10 male guinea-pigs, followed by a topical induction of 25% and a challenge exposure of 12% prothioconazole. Minimal skin irritation was scored in just 1 animal 24 and 48 hours after challenge. A response of only 10% (1/10 animals) indicates that prothioconazole is not a skin sensitiser under the conditions of this study.

A newly-submitted mouse LLNA was conducted according to OECD test guideline 429, but modified to allow cell proliferation to be measured by cell counting rather than radioactive labelling and including the addition of ear swelling measurements. The comparison of the acute reaction (ear weights) with the specific immune reaction (lymph node weights & 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 were administered to groups of six mice. On day 4 of the experiment the mice were sacrificed and the lymphatic organs removed. The weights of the lymph nodes were measured and the cell counts per ml of crushed lymph node were determined.

Cell count results

Concentration (%)	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 simulation index was calculated by dividing the weights/cell counts by that of the controls. The criterion for a positive response in this study with this strain of mouse was a simulation index of greater than 1.4. This was not reached in any dose group; ear swelling and ear weight showed no increase when compared with controls. Therefore, under the conditions of this study prothioconazole was not a skin sensitiser.

10.7.2 Comparison with the CLP criteria

In a GPMT with prothioconazole, only 10% of animals responded; this was below the guidance value of responses in ≥ 30 % of animals in an adjuvant test that would lead to classification. A negative result was also obtained in a mouse local lymph node assay. Therefore no classification for skin sensitisation is proposed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Not classified – Conclusive but not sufficient for classification

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 30*. 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**.

10.8 Germ cell mutagenicity

The genotoxic potential of prothioconazole has been investigated in five *in vitro* studies, covering the end-points bacterial- and mammalian-cell mutation, clastogenicity and aneugenicity, and in three *in vivo* assays (an unscheduled DNA synthesis assay in rat liver and two mouse micronucleus tests).

Table 31: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**
Bacterial point mutation assay (Ames test) OECD 471 (1997) GLP Anonymous, 1996f	Prothioconazole (purity 99.5 %) Solvent: DMSO	Test system: *S. typhimurium* strains TA1535, TA100, TA1537, TA98 and TA102. Concentrations tested: 1.6 - 5000 µg/plate and 1.6 - 500 µg/plate (± S9)	**Negative** (± S9) ≥ 50 µg/plate: marked bacteriotoxic effect (therefore concentrations for the second experiment were lowered)
Mammalian cell mutation assay (V79-HPRT assay) OECD 476 (1984 and 1997) GLP Anonymous, 1996e	Prothioconazole (purity 99.8 %) Solvent: DMSO	Test system: Chinese hamster lung cells (V79) Concentrations tested: - S9: 5, 25, 50, 100, 125, 150, 175 µg/mL + S9: 75, 100, 125, 150, 200 µg/mL (Concentration selection based on two cytotoxicity pre-tests)	**Negative** (± S9) 150 µg/mL + S9: Significant increases in mutant frequency in one culture (2nd assay) but concurrent with extreme cytotoxicity and effect not reproducible in second culture or first assay No increases in mutant frequency in any culture - S9
Mammalian chromosome aberration test OECD 473 (1983; complies also with the 1997 guideline) GLP Anonymous, 1996g	Prothioconazole (purity 99.8 %) Solvent: DMSO	Test system: Chinese hamster lung cells (V79). Concentrations tested: 1st assay: 18 h harvest time: 25, 50, 75*, 100*, 150* µg/mL (± S9) 30 h harvest time: 75, 100, 150* µg/mL (± S9) 2nd assay: 8 h harvest time: 75, 100, 150 µg/mL (± S9) 18 h harvest time: 50*, 75*, 100* µg/mL (- S9) * dose levels examined for chromosomal aberration (dose selection based on cytotoxicity pre-test)	**Positive** (± S9) only at highly cytotoxic concentrations 1st assay: 150 µg/mL ± S9: marked increase in number of cells with aberrations (both 18 and 30 h harvest) 75 and 100 µg/mL - S9: small increase in number of cells with aberrations (both 18 and 30 h harvest) but considered equivocal owing to small magnitude and absence of dose-response relationship 2nd assay: ≥ 50 µg/mL - S9: increase in number of cells with aberrations (18 h harvest) but concurrent with cytotoxicity
In vitro micronucleus assay in human lymphocytes OECD487 (2016) GLP Anonymous, 2017	Prothioconazole (purity 97.6 %) Solvent: DMSO	Test system: human peripheral lymphocytes from 3 donors Concentrations tested: 1st assay (4-hour exposure) 5.6 to 800 µg/ml (+/- S9) 2nd assay (20-hour exposure) 4.7 to 180 µg/ml (-S9) 19.7 to 110 µg/ml (-S9)	**Negative** 1st assay: cytotoxicity from 119 µg/ml –S9 and 79 µg/ml + S9 precipitation from 400 µg/ml 2nd assay: cytotoxicity from 70 µg/ml 1000 binucleated cells / culture evaluated for micronuclei No increase in micronuclei at any

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**
			concentration in either assay
Unscheduled DNA synthesis assay OECD 482 (1986) GLP Anonymous, 1998d	Prothioconazole (purity 99.7 %) Solvent: DMSO	Test system: primary rat hepatocytes Concentrations tested: 1st assay: 1, 5, 10, 12.5, 15, 20, 40 µg/mL 2nd assay: 0.5, 5, 7.5, 10, 12.5, 15, 20 µg/mL (dose selection based on a cytotoxicity pre-test)	**Negative** All nuclear net grain counts (NNG) were below the performing laboratory's criteria for a positive response & without a concentration-related relaionship

h: hours

Table 32: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**
Unscheduled DNA synthesis assay OECD 486 (1997) GLP Anonymous, 1999c	Prothioconazole (purity 99.5-99.7 %) Vehicle: 0.5 % aqueous Cremophor	Test organism/strain: Wistar rats, (Crl:(WI)BR) males, 4/group Dose levels: 0, 2500, 5000 mg/kg bw by gavage (single doses)	**Negative** 5000 mg/kg bw: increase in NNG counts after 16 h in only 2/4 animals but neither group mean nor these individual NNG counts exceeded threshold for a positive response.
Micronucleus assay (*in vivo* mouse bone marrow) OECD 474 (1983) GLP Anonymous. 1996d	Prothioconazole (purity 99.9 %) Vehicle: 0.5 % aqueous Cremophor	Test organism/strain: Albino mice (Hsd/Win:NMRI) males and females 5/sex/group Dose levels: 0 and 250 mg/kg bw by intraperitoneal injection (single doses)	**Negative** 1000 polychromatic erythrocytes per animal were scored for micronuclei. No significant increase in incidence of micronucleated PCEs over vehicle controls at any time point. Clinical signs indicated systemic exposure.

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**
Micronucleus assay (*in vivo* mouse bone marrow) OECD 474 (1997) GLP Anonymous, 2003b	Prothioconazole (purity 95.7 %) Vehicle: 0.5 % aqueous Cremophor	Test organism/strain: Albino mice (Hsd/Win:NMRI) males, 5/group Dose levels: 0, 50, 100, 200 mg/kg bw/d, 2 doses - 24 h apart (total doses: 0, 100, 200, 400 mg/kg bw) by intraperitonieal injection	**Negative** ≥ 50 mg/kg bw: clinical signs indicated systemic exposure 200 mg/kg bw: altered PCE/NCE ratio provided evidence that exposure of the bone marrow occurred 2000 polychromatic erythrocytes per animal were scored for micronuclei. No indications of a clastogenic effect

h: hours
NNG: nuclear net grain
NCE: normochromatic erythrocytes
PCE: polychromatic erythrocytes

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxic potential of prothioconazole has been investigated in several *in vitro* and *in vivo* studies.

Prothioconazole was negative when tested up to cytotoxic concentrations in a battery of *S. typhimurium* strains (TA 1535, TA 100, TA 1537, TA 98, TA 102) in an Ames test. When tested in an HPRT locus mammalian cell mutation assay in V79 cells, significant increases in mutation frequency were recorded in the presence of S9. However, the increases were not reproducible, occurred at extreme cytotoxicity, did not show a dose-response relationship and/or were within the historical control range; hence, the dossier submitter concludes an overall negative result from this study. Prothioconazole was negative in an *in vitro* rat liver UDS assay.

Prothioconazole induced chromosome aberrations in Chinese hamster lung cells in the presence and absence of metabolic activation, but only at highly cytotoxic concentrations. In an *in vitro* micronucleus test in human lymphocytes, conducted in accordance with the most recent (2016) OECD test guideline, prothioconazole did not induce micronuclei when tested up to cytotoxic concentrations. The dossier submitter therefore concludes that prothioconazole is not clastogenic or aneugenic *in vitro*.

In an *in vivo* rat liver UDS assay, an increase in NNG counts was recorded at the top dose after 16 hours, but only in 2 out of 4 animals. The mean NNG counts for the group and the individual counts for these two animals did not exceed the threshold for a positive response applied by the performing laboratory. The dossier submitter therefore concludes that this study was negative.

Two *in vivo* mouse bone marrow micronucleus assays were available. In the first of these, a single dose of prothioconazole (of very high purity = 99.5-99.9%) was administered by intra-peritoneal injection. Clinical signs of toxicity comprised apathy, semi-anaesthetised state, staggering gait, sternal recumbency, spasm and difficulty in breathing. Prothioconazole did not induce a significant increase in the incidence of micronuclei. In the second assay, technical prothioconazole that was representative (in terms of overall purity and impurity profile) of material which was likely to be produced commercially was administered twice, at 24-hour intervals, by intra-peritoneal injection. Prothioconazole was not clastogenic in this assay. Bone-marrow exposure was demonstrated by the altered PCE / NCE ratio, whole-body autoradiography (see section 9) and inferred from the systemic toxicity (apathy, roughened fur, sternal recumbency, spasm, twitching, difficulty in breathing) at doses of ≥ 50 mg/kg bw/d.

10.8.2 Comparison with the CLP criteria

Prothioconazole did not induce mutations in bacterial or mammalian cells *in vitro*, nor did it induce DNA damage as measured by unscheduled DNA synthesis in rat liver cells *in vitro* or *in vivo*. Chromosome aberrations were induced in mammalian cells following incubation with prothioconazole *in vitro*, but only at

highly cytotoxic concentrations. No such effect was noted in an *in vitro* micronucleus test in human lymphocytes nor in two *in vivo* micronucleus tests in mice at doses that caused systemic toxicity and in which bone marrow exposure was either inferred or demonstrated. Overall, therefore, it is concluded that prothioconazole was not genotoxic *in vivo*.

No information is available on the genotoxicity of prothioconazole in humans. Therefore, it clearly does not meet the criteria for classification in category 1A. Since prothioconazole was negative in *in vivo* tests in mammals and there is no information on its mutagenicity in germ cells, classification in category 1B is not appropriate.

Classification for germ cell mutagenicity category 2 may be considered on the basis of positive somatic cell mutagenicity tests *in vivo*, in mammals; or other positive *in vivo* somatic cell genotoxicity tests that are supported by positive results from *in vitro* mutagenicity assays; or positive *in vitro* mammalian mutagenicity assays for substances that also show chemical structure activity relationship to known germ cell mutagens. Since none of these conditions was met, classification in category 2 is not appropriate.

No classification for germ cell mutagenicity is proposed.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified – conclusive but not sufficient for classification

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**.

10.9 Carcinogenicity

The chronic toxicity and carcinogenic potential of prothioconazole has been investigated in two long-term toxicity/carcinogenicity studies in rats (one- and two-year studies) and an 18-month carcinogenicity study in mice.

Table 33: Summary table of animal studies on carcinogenicity

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
1-year rat study Oral (gavage) OECD 452 (1981) GLP Prothioconazole (purity 98.8-99.4%) Vehicle: Aqueous 0.5% Tylose	Rat, Wistar 20/sex/group	0, 5, 50, 750 mg/kg bw/day Daily for 53 weeks	<u>*Non-neoplastic effects*</u> <u>750 mg/kg bw/day</u> 3 deaths (no obvious cause identified); 2 additional deaths attributed to gavage errors Increased volume of urination, ↑ incidence of salivation, bloody muzzle Increased incidence of eyes with water cleft in anterior cortex of lens (females)

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
solution Anonymous, 2000a			↓ body-weight gain (from 13 wks up to 14% lower than controls at termination), ↑ food consumption ↑ water consumption (84% increase in males & 45% increase in females) ↑ liver (mainly females) & kidney (mainly males) weights ↑ macroscopic findings in kidneys Bile duct hyperplasia (females), granular hepatocytes, chronic progressive nephropathy (↑ severity in males, ↑ incidence & severity in females) <u>50 mg/kg bw/day</u> No treatment-related findings <u>5 mg/kg bw/day</u> No treatment-related findings <u>*Neoplastic findings*</u> No increase in any tumour-type at any dose level
2-year rat study Oral (gavage) OECD 451 (1981) GLP Prothioconazole (purity 98.5-99.1 %) Vehicle: aqueous 0.5 % Tylose solution Anonymous, 2001c	Rat, Wistar, Hsd Cpb:WU 50/sex/group	0, 5, 50, 750 mg/kg bw/day 750 mg/kg bw/day reduced to 500 mg/kg bw/day (wk 84, males) and 625 mg/kg bw/day (wk 56, females)	<u>*Non-neoplastic effects*</u> <u>750 mg/kg bw/day</u> ↑ deaths despite dose reduction (26 % survival at termination in males) ↓ BWG (towards end of study: up to 20 % lower than controls), ↓ group mean BW from wk 78 (males) ↑ urine excretion, ↑ emaciation, poor general condition (both sexes), pallor and bloody muzzle (males) ↑ eyes with water cleft in anterior cortex of lens (females) ↑ food consumption, ↑ cumulative food consumption (rel. to BW) ≈15%, ↑ water consumption, ↑ cumulative water consumption (double in males; ≈50% in females); ↓ RBC, ↓ HB, ↓ Hct; in males only: ↑ platelets, ↑ neutrophils and ↑ WBC; ↓ ALT, ↑ ALP, ↓ T4; males only: ↓ glucose, ↓ protein, ↓ albumin, ↑ urea, ↑ creatinine and ↑ cholesterol ↑ urinary volume, ↓ pH, yellow brown crystalloid structures in urine sediment ↑ relative liver weight (25% / 26% in males/females compared with control), ↑ relative kidney weight (+30%/+11% in males/females compared with control) Stomach, liver, lungs – discoloured areas (mainly in males); kidney – surface changes (incl. cysts and discolouration); urinary bladder wall – thickened; salivary glands – oedematous; testes – consistency changes/flaccid; seminal vesicles – reduced in size; rectum – content change; caecum – content change and dilation; pancreas – dilation

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
			Liver – centrilobular hepatocellular hypertrophy with cytoplasmatic change (males: 21/50; females: 33/50), eosinophilic/clear cell foci with cystoplasmic change (males: 9/50; females: 16/50) Kidney – increased mean severity of chronic progressive nephropathy (males)(4.4; control: 2.5) Urinary bladder – increased incidence of transitional cell hyperplasia (males: 8/50; females: 8/50) Thyroids – no notable histopathological findings <u>50 mg/kg bw/day</u> ↑ urine excretion (males) ↑ platelets (males) ↑ ALP (females), ↓ T4 (marginally) Liver – centrilobular hepatocellular hypertrophy with cytoplasmatic change (males only; 10/50) Kidney – ↑ severity of chronic progressive nephropathy in males <u>5 mg/kg bw/day</u> No findings <u>*Neoplastic findings*</u> No notable neoplastic findings (no increases at any dose) in the liver, kidneys or urinary bladder, nor in any other organs or tissues at any dose
18 month mouse study Oral (gavage) OECD 451 (1981) GLP Prothioconazole (purity 98.2-98.9 %) Vehicle: aqueous 0.5 % Tylose solution Anonymous, 2001d	Mouse, CD-1 60/sex/group	0, 10, 70 and 500 mg/kg bw/day	<u>*Non-neoplastic effects*</u> <u>500 mg/kg bw/day</u> ↓ BWG, ↓ term. BW (≈10 %) ↑ liver weight (absolute and relative; > 20 %), ↓ kidney weight (absolute and relative), sign. ↓ uterus weight (absolute and relative; partially secondary to lower terminal BW) Liver – ↑ distinct lobulation (5/60 males); kidney – changes to the surface and color Liver – centrilobular hepatocellular hypertrophy/fine granular eosinophilic change (males: 48/60; females: 33/60) Kidney – tubular degeneration/regeneration (males: 50/60; females: 37/60), subcapsular tubular degeneration/fibrosis (males: 34/60; females: 27/60) <u>70 mg/kg bw/day</u> ↓ BWG ↑ liver weight (absolute and relative; ≥ 10 %) Liver – ↑ distinct lobulation (2/60 males); kidney – changes

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	Results
			to the surface Liver – centrilobular hepatocellular hypertrophy/ fine granular eosinophilic change (31/60 males) Kidney – tubular degeneration/regeneration (33/60 males) 10 mg/kg bw/day No treatment-related effects *Neoplastic findings* No increase of neoplastic findings in either liver or the kidneys despite treatment-related effects in these organs No indication of a treatment-related effect in pattern of neoplastic findings in any organs or tissues – no increase in any tumour at any dose group

↑ / ↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.
BW: Body weight BWG: body-weight

10.9.1 Chronic/carcinogenicity studies in rats

The chronic toxicity and carcinogenicity of prothioconazole has been investigated in one- and two-year oral studies in rats.

In the one-year chronic toxicity study, prothioconazole was administered by gavage at doses of 0, 5, 50 and 750 mg/kg bw/day. Five animals at 750 mg/kg bw/day were found dead or were sacrificed moribund (three males, two females). Two of the deaths were attributed to gavage errors, but the study authors were not able to identify a reason for the other three deaths (and one of these animals showed autolysis). The applicant for renewal of this active substance under Regulation 844/2012 has surmised that they resulted from kidney failure, owing to indications of kidney toxicity at this dose: increased water consumption, increased urinary excretion, urinalysis findings and kidney histopathology. However, the pathology data do not allow a definitive conclusion on the cause of death to be made. Nevertheless, the kidney was a clear target organ of prothioconazole at this dose. The (histo)pathological findings in the kidneys at 750 mg/kg bw/d (roughened surface, increased incidence or severity of chronic progressive nephropathy) were supported by urinalysis findings. Increased water consumption was most likely linked to the kidney effects and associated increase in urination. There were no treatment-related tumours in this study after a one-year exposure.

The same doses were then administered in the two-year study. The longer duration of exposure led to a clear exceedance of the maximum tolerated dose at 750 mg/kg bw/d, with an increased number of deaths and emaciation. Consequently, this dose was reduced to 500 mg/kg bw/day in males from week 84 and to 625 mg/kg bw/d in females from week 56. Despite this, the number of deaths in high-dose males continued to be higher than in the other groups during the rest of the study; in contrast, after the dose was reduced the mortality rate in high-dose females was similar to the controls. The increased number of deaths in the high-dose males resulted in a survival of < 50 % in this group at the termination of the study (26 % survival). Survival in all the other groups exceeded 50 % at study termination (see table below).

Parameter	Mortality (%) animals treated at (mg/kg bw/day):							
	Male				Female			
	0	5	50	750/500	0	5	50	750/625
Week 56	2	4	4	6	6	4	4	22
Week 85	6	12	18	32	20	24	16	28

Week 106	38	36	34	74***	46	36	30	46
Survival at study termination (%)	31/50 (62 %)	32/50 (64 %)	33/50 (66 %)	13/50 (26 %)	27/50 (54 %)	32/50 (64 %)	35/50 (70 %)	27/50 (54 %)

The low survival in the males at 750 mg/kg bw/d could have reduced the sensitivity of the study to detect treatment-related tumours. However, survival in this group fell below 50 % late in the study (at almost 22 months) and all decedents were examined for tumours. There was no indication of an increase in tumour incidences or a decreased latency in decedents or animals that survived to termination. The occurrence of some toxicity in mid-dose males (slightly increased severity of chronic progressive nephropathy; urinary effects; increased platelets) but without neoplastic findings provided reassurance that the study was not compromised by the reduced survival to termination of the high-dose males. Furthermore, survival of the high-dose female group was satisfactory. The dossier submitter concludes, therefore, that the study was adequate for the detection of a carcinogenic potential of prothioconazole.

The increase in deaths in the male high-dose group, even after reduction of the dose, was attributed by the applicant for renewal under Regulation 844/2012 to the adverse kidneys effects. At 750 mg/kg bw/d, effects on the kidneys and urinary tract were characterised by markedly increased water consumption, urinalysis effects, increased kidney weights, crystalline material in urine sediment (not identified), increased severity of chronic progressive nephropathy and transitional cell hyperplasia in the urinary bladder. At the mid-dose level, 50 mg/kg bw/d, the severity of chronic progressive nephropathy was slightly increased in males and urinary output was also increased throughout the study.

Liver toxicity was recorded at 750 mg/kg bw/d in both studies. The effects included increased liver weights, histological evidence of enzyme induction, and changes in clinical-chemistry parameters that indicated liver damage. A higher incidence of bile duct hyperplasia was also reported in the high-dose females in the one-year study, whilst eosinophilic/clear cell foci with cytoplasmic change was noted after two-years of administration. At 50 mg/kg bw/d, liver effects mainly comprised adaptive changes. Liver-enzyme induction was likely to be responsible for the reductions in T4 levels that was observed in both studies, but without any consequences on thyroid weights or (histo)pathology.

At 750 mg/kg bw/d, the number of eyes of females with water clefts in the anterior cortex of the lens was increased at one year in both studies. There were no histopathological correlates for this finding. At two years, the incidence in the control females had reached the same level as that in the high-dose females. The study authors stated that cortical water clefts are known precursors of lens cataracts and occur in rats of this age with a relatively wide variation. Therefore, this finding is considered to be a treatment-related exacerbation of an age-related lesion that was possibly secondary to the overall toxicity in this group.

Despite the hyperplastic changes recorded in organs and tissues associated with the urinary tract, there was no increase in neoplastic findings in these organs and tissues in the two-year study. Similarly, there were no notable neoplastic findings in the liver or thyroid, nor in any other organ or tissue. The overall incidence of tumour-bearing animals, the time of occurrence and the pattern of neoplastic findings did not indicate a carcinogenic effect.

In conclusion, therefore, prothioconazole was not carcinogenic in rats in these studies when tested up to a dose that was clearly toxic.

10.9.2 Chronic/carcinogenicity study in mice

The carcinogenic potential of prothioconazole in mice was investigated in an 18-month study *via* the oral route of administration, in which groups of 60/sex CD-1 mice received daily gavage doses of 0, 10, 70 and 500 mg/kg bw/day.

Survival to termination was similar and exceeded 50 % in all groups: 85 %, 95 %, 88 %, 77 % in males and 82 %, 85 %, 82 %, 78 % in females at 0, 10, 70 and 500 mg/kg bw/day, respectively. Clinical signs were observed only in the high-dose group (piloerection, pallor and poor general condition); these occurred near to the end of the study. Lower body weight gains were recorded at 500 and 70 mg/kg bw/day after 4-6 weeks of

treatment, although only females from the high-dose group had terminal body weights > 10 % lower than controls. There was no effect on food consumption.

The target organs were the liver and kidneys. At 500 mg/kg bw/d, liver weights were statistically significantly increased (relative by 39 % in males and females), whilst kidney weights were decreased in males (relative by 13 %). Pathology findings in the liver comprised lobulation in males and indications of hepatic enzyme induction (hypertrophy with cytoplasmic change) in both sexes. In the kidneys, roughened surfaces were reported in males, together with an altered kidney colour in both sexes (although dose-response relationships were not evident). Histopathological examination of the kidneys revealed an increased incidence of tubular degeneration / regeneration and subcapsular degeneration / fibrosis in both sexes. Decreased absolute and relative uterine weights were partially explained by the decrease in terminal body weight, and in the absence of (histo)pathological correlates are concluded by the dossier submitter not to represent an adverse effect.

At 70 mg/kg bw/d, relative liver weights were increased by 16 % in males (potentially adverse) and 10 % in females; in males, this correlated with histopathological indications of liver-enzyme induction (hypertrophy and cytoplasmic change; no findings in females). There was no change in kidney weight at this dose. Upon histopathology, an increased incidence of renal tubular degeneration / regeneration was evident in males. No adverse effects were observed at 10 mg/kg bw/d.

There were no increases in any neoplastic findings in the liver or kidneys, nor in any other organs or tissues, in any dose group. The total number of tumours was lower in the high-dose groups than the controls. It is concluded that prothioconazole was not carcinogenic in mice in this study. The combination of the adverse body-weight effects and the high incidence of histopathological findings in the kidneys suggest that the high-dose level approached the maximum tolerated dose and thus was sufficiently high.

10.9.3 Short summary and overall relevance of the provided information on carcinogenicity

The carcinogenic potential of prothioconazole was investigated in three long-term oral toxicity/carcinogenicity studies, two in the rat and one in the mouse.

The highest doses administered, 750 mg/kg bw/d in rats (reduced in the two-year study to 500 mg/kg bw/d and 625 mg/kg bw/d in males and females, respectively because of excessive toxicity) and 500 mg/kg bw/d in mice, approached or exceeded the maximum tolerated dose and were therefore suitable for the assessment of prothioconazole's carcinogenic potential. Although, in the two-year rat study, survival of the high-dose males was adversely affected, the dossier submitter has concluded that the validity of the study was not compromised: survival in this group fell below 50 % late in the study; the mid-dose level induced some toxicity but without neoplastic findings; and survival of the high-dose female group was satisfactory. Survival in the mouse study exceeded 50 % in all groups.

The kidneys / urinary tract and liver were the target organs in both species. Liver-enzyme induction was likely to be responsible for the decreased T4 in both the rat studies (not measured in mice). Despite these effects, increased tumour incidences were not recorded in either the kidneys / urinary tract, liver or thyroid (nor were any (histo)pathological effects observed in the latter). There were no increases in tumours in any organ or tissue in either species.

In conclusion, prothioconazole was not carcinogenic in rats or mice in the available studies.

10.9.4 Comparison with the CLP criteria

Long-term toxicity/carcinogenicity studies in rats (one- and two-years) and mice (18-months) were conducted to assess the carcinogenic potential of prothioconazole. There was no evidence that prothioconazole was carcinogenic in either species in these studies. Thus a classification for carcinogenicity is not required for prothioconazole.

10.9.5 Conclusion on classification and labelling for carcinogenicity

Not classified – Conclusive but not sufficient for classification

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**.

10.10 Reproductive toxicity

The reproductive toxicity of prothioconazole has been investigated in rats and rabbits. A two-generation study in rats, supplemented with a range-finding preliminary study, is available to investigate the effects of prothioconazole on sexual function and fertility. Three developmental toxicity studies in rats (two oral and one dermal) and one in rabbits (oral) are also available.

10.10.1 Adverse effects on sexual function and fertility

The effect of prothioconazole on sexual function and fertility has been investigated in a range-finding preliminary reproductive study and a two-generation reproduction study in rats.

Table 34: Summary table of animal studies on adverse effects on sexual function and fertility

Study, species (strain)	Dose levels	Critical effects
Range-finding reproductive study Oral (gavage) Non guideline GLP Rats, Wistar Hannover 10/sex/group Prothioconazole (purity 98.1-98.8%) Vehicle:0.5% aqueous methylcellulose/Tween 80 Anonymous (1999d)	0, 10, 100, 250 & 500 mg/kg bw/day From 4 weeks before mating until 21 days post-partum	No parental deaths <u>500 mg/kg bw/d</u> Urine staining of fur (1 male/3 females during pre-mating/mating and 2 females during gestation) ↓ paternal body weights (minimal) ↓ pup body weights from PND4 (↓ by 7-10 %, not statistically significant) <u>250 mg/kg bw/d</u> No treatment related effects <u>100 mg/kg bw/d</u> No treatment related effects <u>10 mg/kg bw/d</u> No treatment related effects
Two-generation study in rats Oral (gavage) OECD 416 (2001) Deviations: thyroids not weighed in adults/ morphological examination of sperm samples for P generation not performed. GLP Rats, Wistar Hannover, Crl:WI(Han) 30/sex/group Prothioconazole (purity 98.1-98.8 %) Vehicle: 0.5 % aqueous methylcellulose / Tween 80 Anonymous, 2001e	0, 10, 100 and 750 mg/kg bw/d From 10 weeks pre-mating to day 21 post-partum	**Parental toxicity** **F0 generation** <u>750 mg/kg bw/d</u> Urine stain (4/30 males, 17/30 females), salivation (4/30 males, 5/30 females), dehydration (2/30 males, no females) ↓ BW during pre-mating (males, 7%), marginal ↓ BW gain during gestation (females, -3 %) ↑ Food consumption during pre-mating (up to 19% males) with ↓ food efficiency, slightly ↓ food consumption during lactation (females) ↑ liver weight (males & females, relative > 18 %), ↑ kidney weight (males, relative 15 %), ↓ thymus weight (females, relative 29 %) Hepatocytomegaly (28/30 males, 1/30 controls; 4/30 females, none in controls), multifocal cortical nephrosis (27/30 males and 4/30 females, none in controls) <u>100 mg/kg bw/d</u> ↑ liver weight (males, relative by 7 %) ↓ thymus weight (females, relative by 20 %) <u>10 mg/kg bw/day</u> No adverse effects **F1 Generation** <u>750 mg/kg bw/d</u> Urine stain (4/30 males, 4/30 females), salivation (3/30 females), dehydration (1/30 males, 1/30 females)

		Marginally ↓ BW gain (-5 %) during gestation (females) ↑ Food consumption during pre-mating (up to 28 %, males & females) with reduced food efficiency, slightly ↓ food consumption during lactation (females, up to 9 %) ↑ liver weight (males & females, relative up to 24 %), ↑ kidney weights (males, relative by 21 %) Hepatocytomegaly (27/30 males compared with 3/30 controls; 20/30 females compared with 1/30 controls), multifocal cortical nephrosis (30/30 males and 6/30 females, none in controls) <u>100 mg/kg bw/d</u> ↓ BW during pre-mating (8%, males only; initial body-weights lower than controls) ↑ liver weight (females, relative by 9 %) <u>10 mg/kg bw/day</u> No adverse effects
		Fertility
		<u>750 mg/kg bw/d</u> ↓ no. of oestrous cycles (2.7 (F0) & 3.1 (F1) compared with 3.4 & 3.6 in controls), corresponding to an ↑ in cycle duration at 750 mg/kg bw/day (5.1(F0) & 4.7(F1) days, compared with 4.3 & 4.4 in controls) ↓ implantation sites (10.8(F0) & 9.3(F1) compared with 11.8 & 10.7 in controls) (not statistically significant) ↓ litter size (10.0 (F0) & 8.2 (F1) compared with 10.8 & 10.2 in controls) (not statistically significant) Slightly ↑ duration of gestation (22.3 & 22.4 days compared with 21.9 & 22.0 in controls) ↑ days to insemination in F1 generation (3.8 days compared with 2.4 in control) (not statistically significant) No effects on mating, fertility or gestation indices at any dose. No effects on any parameter at 10 and 100 mg/kg bw/d
		Offspring toxicity

		F1 pups 750 mg/kg bw/d Salivation prior to dosing, urine stain (3 pups from 1 litter) ↓ BW gain (reduced by 9 to 17 %) from PND4 onwards ↓ absolute and relative spleen weight ↑ no of days to preputial separation in males (delayed by 2.5 days on average - attributed to retarded growth) 100 mg/kg bw/d No adverse effects 10 mg/kg bw/d No adverse effects **F2 pups** 750 mg/kg bw/d ↓ BW gain (reduced by 6 to 12 %) from PND7 onwards ↓ absolute and relative spleen weight ↑ anogenital distance in males and females (attributed to slightly higher birth weight resulting from slightly longer duration of gestation in F1 dams) 100 mg/kg bw/d No adverse effects 10 mg/kg bw/d No adverse effects

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a preliminary reproduction study, parental toxicity was suggested at the highest dose tested (500 mg/kg bw/d) by the observed urine stains, which are interpreted as a sign of disturbance of kidney function and systemic water / electrolyte homeostasis. Reproduction was not affected at any dose. Pup body weights were slightly (not statistically significantly) decreased at 500 mg/kg bw/d during lactation; in view of the pup body-weight effects in the two-generation study (see below), the dossier submitter considers it prudent to regard this finding as a possible treatment-related effect.

In a two-generation study, prothioconazole was administered daily via gavage to groups of 30/sex Wistar rats at doses of 0, 10, 100 and 750 mg/kg bw/day from 10-weeks before mating, during the two-week mating period, throughout gestation and lactation and up to 21 days post-partum (weaning of F_1 pups). Selected F_1 progeny then received similar treatment until weaning of the F_2 generation.

Parental toxicity

The parental toxicity at 750 mg/kg bw/d primarily comprised effects that indicated kidney dysfunction, namely urine stains and dehydration in both generations. No clinical signs were evident at 10 or 100 mg/kg bw/d. Water consumption was not measured in this study, but food efficiency was reduced and body-weights decreased in males at 750 mg/kg bw/d, with slight but statistically significant reductions in body-weights in males also at 100 mg/kg bw/d. In females, effects on body-weight were less marked than in males, with only marginal changes observed at 750 mg/kg bw/d during gestation and lactation, none of which was statistically significant. Feed consumption at this dose was slightly decreased during lactation.

In both generations, absolute and relative kidney weights were increased at 750 mg/kg bw/d in males only, whilst absolute and relative liver weights were increased from 100 mg/kg bw/d (males and females). Absolute and relative thymus weights were decreased in F0 females at 100 and 750 mg/kg bw/d, but since this was without any pathological correlates, this change did not represent an adverse effect.

At 750 mg/kg bw/d, histopathological examinations revealed an increase in the incidence of hepatocytomegaly in male and female livers and multifocal cortical nephrosis in male and female kidneys (more pronounced in males). No notable histopathological changes were observed at dose levels of 10 or 100 mg/kg bw/day in either generation.

Fertility

Mating, fertility and gestation indices were not affected by treatment at any dose level in either generation. Slight differences in the number of days to insemination (F_1 generation at 750 mg/kg bw/day) and duration of gestation (both generations at 750 mg/kg bw/day) were not statistically significant. Reduced numbers of implantation sites and reduced mean litter sizes in both generations at the high-dose level were within the historical control data provided for the F_0 generation. All the reproduction parameters were unchanged in the mid- and low-dose groups.

Reproductive data of F_0- and F_1-generation animals

Parameter	Prothioconazole (mg/kg bw/d)				Historical control data [a]
	0	10	100	750	
F_0-Generation					
No. mated / no. paired	27 / 30	30 / 30	30 / 30	30 / 30	197/200
No. delivering a litter	24	30	29	28	173
No. with implants	24	30	29	28	176
Mating index	90.0	100	100	100	90.0-100.0
Fertility index	88.9	100	96.7	96.7	86.2-96.6
Gestation index	100	100	100	96.6	96.0-100.0
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. 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
F_1-Generation					
No. mated / no. paired	30 / 30	30 / 30	30 / 30	29 / 30	149/150
No. delivering a litter	27	26	28	26	132
No. with implants	27	26	28	26	133
Mating index	100	100	100	96.7	96.7-100.0
Fertility index	90.0	86.7	96.7	93.1	75.9-96.7
Gestation index	100	100	96.6	92.6	95.5-100.0
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. 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

Mating index = no. inseminated / no. paired x 100; Fertility index = no. pregnant / no. inseminated x 100
Gestation index = no. with live pups/ no. pregnant x 100
[a] Historical control range from 7 studies in Wistar rats performed 1998-2001
* $p < 0.05$ (Kruskal-Wallis and Dunn's Test)
Findings considered related to treatment with prothioconazole are written in **bold letters**

There were no treatment-related changes in sperm parameters. A decrease in the number of oestrus cycles and concomitant increase in cycle length occurred at 750 mg/kg bw/day that was more pronounced in the F_1 generation than the F_0 generation. An examination of primordial ovarian follicles of 10 females per group failed to establish a dose-response relationship, nor was there a dose-related, consistent change in corpora lutea

counts. Overall, taking into account the absence of adverse effects on mating and fertility indices, the dossier submitter concludes that the slight changes in oestrus cycling were most likely to be secondary to the maternal toxicity in the high-dose group and not biologically important.

Offspring toxicity

Offspring toxicity was restricted to the high-dose group. Clinical signs consisted of salivation prior to dosing and urine stains. Pup weight-gain was statistically significantly retarded at the high-dose level in both generations from either day 4 or day 7 *post-partum*; there were no differences from the controls in pup weight at birth. At necropsy, reduced spleen weights were also apparent at this dose. In the mid- and low-dose groups, pup growth was similar to the controls. Parameters of pup viability (birth index, live-birth index, viability index, lactation index) were unaffected by administration of prothioconazole at all dose levels.

A slight delay in preputial separation in F1 pups at 750 mg/kg bw/day was attributed by the study authors to the retarded growth of the pups at this dose, which was itself secondary to general systemic toxicity. An analysis of the body weight of the pups on the individual days of preputial separation demonstrated a clear link between body weight and the day of preputial separation. In the F2 generation, a slightly greater anogenital distance at birth in male and female high-dose pups (within the historical control data) was clearly correlated with a higher body-weight at birth, which the study authors attributed to a slightly longer duration of gestation in the these dams. The dossier submitter agrees that these minor findings were secondary to general systemic toxicity and were not indicators of specific effects on development.

10.10.3 Discussion and comparison with the CLP criteria

The potential of prothioconazole to adversely affect sexual function, fertility and offspring survival has been investigated in a preliminary range-finding study and a guideline-compliant two-generation reproduction study in Wistar rats.

In both studies there was marked parental toxicity in the high-dose groups (500 mg/kg bw/d in the range-finding study; 750 mg/kg bw/d in the main study). Urine stains were common to both studies, and in the main study dehydration (albeit in a small number of animals) was also recorded. Kidney toxicity with severe dehydration and increased water consumption was a feature of prothioconazole administration in several repeated-dose studies in rats at similar doses (sections 10.9 and 10.12) and in the developmental toxicity studies (section 10.10.4), which eventually led to death after prolonged administration for one to two years. Although water consumption was not measured in the reproduction studies, the urine staining was likely to be a sign of effects on the kidney and water homeostasis systems, consistent with the findings of the repeated-dose toxicity studies. Histopathological changes in the kidneys were also observed at 750 mg/kg bw/d in the main study. It is therefore reasonable to surmise that the observed urine stains and dehydration were manifestations of kidney dysfunction; furthermore, cortical nephrosis was observed at this dose. The dossier submitter therefore considers that 750 mg/kg bw/d was a clearly maternally-toxic dose.

Other treatment-related parental findings in the high-dose group included increased food consumption and a decrease in body-weight gain (indicative of a reduction in food utilisation efficiency), increases in organ weights, particularly the liver and kidneys, and histopathological changes in the liver. A slight decrease in body weight was also evident in males at 100 mg/kg bw/d, as was a decrease in thymus weight in females.

With regards to fertility, there were no treatment-related effects on either mating or fertility indices in either generation; slight increases in the time to insemination were not statistically significant. Pregnancy outcome was not affected by exposure to any dose. Slightly reduced mean numbers of implantation sites and litter sizes in the high-dose group were within the historical control data for the F_0 generation and were not likely to be real effects or were a consequence of maternal toxicity, as was the slight increase in the duration of gestation in F1 dams (not associated with clinical signs or deaths in either dams or pups).

Prothioconazole did not adversely affect pre-natal or post-natal pup viability at any dose. Toxicity to pups in both generations consisted of clinical signs (urine staining and salivation) and retarded growth only at 750 mg/kg bw/d, secondary to general offspring toxicity. There was no evidence of a specific effect on development.

In conclusion, in the available studies prothioconazole did not demonstrate a specific effect on reproduction. Minor changes to some parameters occurred only in the high-dose group and were secondary to the relatively severe maternal toxicity that was induced at this dose. Therefore, no classification for adverse effects on sexual function and fertility is warranted.

10.10.4 Adverse effects on development

The potential of prothioconazole to adversely affect development has been investigated in rats and rabbits by the oral route and in rats by the dermal route of administration.

Table 35: Summary table of animal studies on adverse effects on development

Study, species	Dose levels	Critical effects
Developmental toxicity studies in rats		
Dose-range finding study Oral (gavage) Non-guideline Non-GLP Rats, Wistar Females 5/group Prothioconazole (purity not specified) Vehicle: 0.5% aqeous xylose Anonymous, 1995	1000 mg/kg bw/day (days 6-19 post coitum), 300 mg/kg bw/day (days 6-19 post coitum) & 100 mg/kg bw/day (days 6-15 post coitum)	There were no deaths at any dose <u>1000 mg/kg bw/d</u> ↑ water consumption ↑ urination ↓ body weight gain ↑ incidences of supernumerary ribs (12% with 14th ribs) <u>300 mg/kg bw/d</u> ↓ body weight gain ↑ incidence of resorption ↑ incidences of supernumerary ribs (24% with 14th or cervical ribs) <u>100 mg/kg bw/d</u> No treatment-related effects
Developmental toxicity study in rats Oral (gavage) OECD 414 (1981) GLP Rat, Wistar, Hsd Cpb:WU Females 26/group Prothioconazole (purity 99.5-99.8 %) Vehicle: 0.5 % aqueous carboxy-methylcellulose Anonymous, 1997a	0, 80, 500, 1000 mg/kg bw/d on gestation days days 6-19	**Maternal toxicity** <u>1000 mg/kg bw/d</u> ↑ urination (21/26 dams) ↓ BW gain during gestation by 31 % (corrected for uterus weight), transient BW loss (d 6-8: BW gain -154 % compared with control) ↓ food consumption (d 6-11: -17.2 %) ↑ Water consumption by 59-75 %; visually increased in 20/26 dams ↑ Cholesterol , ↓ T4, ↓ T3, ↓ AST, ↑ ALT, ↑ ALP ↑ Relative liver weight 8% Material deposit in ureter and urinary bladder, multiple white areas in kidneys (urolithiasis & hydronephrosis) in 1/26 animals <u>500 mg/kg bw/d</u> ↑ urination (14/26 dams) ↓ BW gain during gestation by 21% (corrected for uterus weight) ↑ Water consumption by 23-31 % (visually increased in 8/26 dams) ↑ Cholesterol, ↓ T4, ↓ T3 <u>80 mg/kg bw/d</u> No adverse effects **Developmental toxicity** <u>1000 mg/kg bw/d</u> *Visceral findings*

<table>
<tr><th>Study, species</th><th>Dose levels</th><th>Critical effects</th></tr>
<tr><td></td><td></td><td>
↑ foetuses with engorged placentae (4.3 %; 0.7 % of controls) but with no corresponding effect on placental weight
↓ Pup weight (both sexes combined: 5 %)
↑ Incidence of dilatation of renal pelvis (foetal (litter) incidence (%): 17.0 (54.2) (control: 7.2 (26.9)); considered secondary to retarded foetal development
↑ Incidence of microphthalmia 4.6% foetal/33.3% litter (0/0 control)

Incidences (%) of microphthalmia summarised in tables below – foetal (litter).
<table>
<tr><td>Dose levels (mg/kg bw/d)</td><td>0</td><td>80</td><td>500</td><td>1000</td><td>HCD range[b]</td></tr>
<tr><td>Microphthalmia[a]</td><td>0 (0.0)</td><td>2.4 (15.4)</td><td>1.1 (13.6)</td><td>4.6 (33.3)</td><td>0-1.95 (0-20)</td></tr>
</table>
[a] Total number of foetuses with microphthalmia (foetuses with microphthalmia at external examination were assigned to the subgroup for visceral examination. Cases of microphthalmia missed at external examination and assigned to skeletal evaluation would be detected as “eyehole reduced in size”. External, visceral and skeletal incidences of microphthalmia are combined to derive an “all foetuses” value (related to all (viscerally and skeletally) examined foetuses) for the purpose of comparison with the historical control data

[b] Data from 1993-99 (26 studies). The same range applies for studies from 1990-2002 (49 studies (26 as before + 23 additional studies from 1990-92 and 2000-02)).

Skeletal findings

↑ incidence of incomplete ossification (distal and proximal phalanges, caudal vertebral bodies and 6th sternebral bone)

↑ incidence of rudimentary ribs (foetal (litter) incidence (%) see table below)
<table>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>80</td><td>500</td><td>1000</td><td>HCD range[c]</td><td>HCD range[d]</td></tr>
<tr><td>Short 14th rib</td><td>0.7 (3.8)</td><td>7.1* (42.3)**</td><td>10.6* (54.5)**</td><td>25.2** (62.5)**</td><td>0-12.2 (0-40)</td><td>0-24.4 (0-57)</td></tr>
</table>
[c] Data from 1993-1999 (24 studies)

[d] Data from 1992-2000 (29 studies (24 studies as above + 5 add. studies from 1992 and 2000))

<u>500 mg/kg bw/d</u>

No adverse effects

<u>80 mg/kg bw/day</u>

No adverse effects
</td></tr>
</table>

<table>
<tr><th>Study, species</th><th>Dose levels</th><th>Critical effects</th></tr>
<tr><td>Range-finding study in Rat, Wistar Hanover, Crl:WI(Han)
Oral (gavage)
Not guideline or GLP
Females, 12/group
Prothioconazole (purity 98.7 %)
Vehicle: 0.5 % aqueous carboxy-methylcellulose
Anonymous, 2004a</td><td>0, 500, 1000 mg/kg bw/d on gestation days 6 to 19</td><td>Maternal toxicity
<u>1000 mg/kg bw/d</u>
Three deaths, resulting from dehydration
↓ body-weight and food consumption
37 % ↑ in water consumption
Clinical-chemistry changes, ↓ in gravid uterine weight
<u>500 mg/kg bw/d</u>
Dehydration in 1 dam
↓ body-weight and food consumption
24 % ↑ in water consumption
Clinical-chemistry changes, ↓ in gravid uterine weight
Developmental toxicity
No external foetal findings at either dose.</td></tr>
<tr><td>Developmental toxicity study in rats
Oral (gavage)
GLP
OECD 414 (2001)
Deviations: no visceral investigations were conducted; study was performed to investigate the findings of microphthalmia and rudimentary 14 rib in the previous study.
Rat, Wistar Hanover, Crl:WI(Han)
Females
25/group
Prothioconazole (purity 98.7 %)
Vehicle: 0.5 % aqueous carboxy-methylcellulose
Anonymous, 2004b</td><td>0, 20, 80, 750 mg/kg bw/d on gestation days 6-19</td><td>Maternal toxicity
<u>750 mg/kg bw/day</u>
↓ Net BW gain d 0-20 (13 %), ↓ BW gain d 6-12 (46 %)
↓ Food consumption d 6-12 (18 %)
↑ Water consumption (up to > 170 % of control)
↑ BUN, ↑ Cholesterol, ↑ ALP, ↓ AST
<u>80 mg/kg bw/day</u>
No adverse effects
<u>20 mg/kg bw/day</u>
No adverse effects
Developmental toxicity
<u>750 mg/kg bw/d</u>
No foetuses with microphthalmia. No compound-related effects on individual or mean eye weight, eye-to-foetal weight ratios or on eye measurements.
Marginal ↑ increase of comma-shaped supernumerary ribs
Supernumerary 14th ribs (foetal (litter) incidence (%))
<table>
<tr><th>Dose levels (mg/kg bw/d)</th><th>0</th><th>20</th><th>80</th><th>750</th><th>HCD range[e]</th></tr>
<tr><td>Rudimentary (punctiform)</td><td>23.5 (95.2)</td><td>18.2 (77.8)</td><td>27.6 (88.9)</td><td>33.6 (95.7)</td><td>19 - 52 (57 - 91)</td></tr>
<tr><td>Rudimentary (comma-shaped)</td><td>11.8 (52.4)</td><td>7.4 (66.7)</td><td>12.4 (38.9)</td><td>21.2* (69.6)</td><td>5 - 18 (9 - 58)</td></tr>
</table>
[e] Data 4 studies (522 foetuses, 97 litters) conducted 1998-2002, same laboratory and strain
No adverse effects at 80 or 20 mg/kg bw/d.</td></tr>
<tr><td>Developmental toxicity study in rats</td><td>1000 mg/kg bw/d (neat)</td><td>Maternal toxicity
No adverse effects at any dose level for neat application and</td></tr>
</table>

Study, species	Dose levels	Critical effects
Dermal OECD 414 (1981) GLP Rat, Wistar Hannover, Crl:WI(HAN) Females 29-30/group Prothioconazole (98.1-98.8 %) & EC 250 formulation (25% prothioconazole) Vehicle: deionised water Anonymous, 2001f	Equivalent to 250 mg/kg bw/day & 62.5 mg/kg bw/day for EC 250 formulation Daily topical applications (6 h/day) from gestation days 6-19	formulation **Developmental toxicity** No adverse effects at any dose level for neat application and formulation
Developmental toxicity studies in rabbits		
Range-finding study in rabbits Oral (gavage) Non-guideline Non-GLP Chinchilla rabbits, CHbb:CH, Hybrids Females 3 or 5/group Prothioconazole (purity 99.7 %) Vehicle: 0.5 % aqueous carboxy-methylcellulose Anonymous, 1997b	Dose levels: 0, 80, 100, 300, 480 mg/kg bw/d from gestation days 6-27	**Maternal toxicity** ≥ 80 mg/kg bw/d 1-2 deaths between d 23 and 27 Body-weight loss ↓ Food consumption **Developmental toxicity** 480 mg/kg bw/d ↓ Foetal weights 1 female with total post-implantation loss

Study, species	Dose levels	Critical effects
Developmental toxicity study in rabbits Oral (gavage) OECD 414 (1981; dosing pattern and foetal examinations in line with the 2001 guideline) GLP Chinchilla rabbits, CHbb:CH, Hybrids Females 24/group (owing to low pregnancy incidence, 6 and 7 additional mated females were added to the 10 and 80 mg/kg bw/d, respectively) Prothioconazole (purity 99.5-99.7 %) Vehicle: 0.5 % aqueous carboxy-methylcellulose Anonymous, 1998b	Dose levels: 0, 10, 30, 80 and 350 mg/kg bw/d from gestation day 6-27	**Maternal toxicity** 350 mg/kg bw/d 1 female died on d 25 ↓ Overall body-weight gain (53 %) / BW loss d 6 - 12 ↓ Food consumption (31 %) **Developmental toxicity** 350 mg/kg bw/d Abortions in 3 females (on d 22, 25 and 27) ↑ Post-implantation losses (3 females with total litter resorption) ↓ Foetal weights (10-13 %) Slightly ↓ placental weight (5.8 %) Differences in foetal incidences of incomplete and absent ossification of one or more sternebrae (↑) and phalanges of the digits (partly ↑ or ↓) and ↓ unossified 13th rib No other notable effects on nature and incidences of external, visceral and skeletal abnormalities were observed. No increase in microphthalmia (only 1 single incidence in the low dose group in a foetus with multiple malformations affecting the head). No adverse effects were noted at 0, 10, 30 and 80 mg/kg bw/day

10.10.4.1 Developmental toxicity in rats

The developmental toxicity of prothioconazole has been investigated in rats in two oral (gavage) studies and one study via the dermal route.

In the first developmental toxicity study, maternal toxicity was observed at the mid- and high-dose levels of 500 and 1000 mg/kg bw/day and was characterised by increased urination and greatly increased water consumption.

Food consumption was reduced at the top-dose level in pregnant females during days 6-11. Statistically significantly reduced body-weight gains during gestation were recorded at isolated time-points at 500 and 1000 mg/kg bw/day, with animals at 1000 mg/kg bw/day showing transient body-weight loss over days 6-8. Cumulative body-weight gain was slightly decreased on days 6-19 and 0-20 at 1000 mg/kg bw/day. Body-weight gain corrected for uterine contents was statistically significantly and dose-dependently decreased (by up to 31 %) in the 500 and 1000 mg/kg bw/day dose groups. At necropsy, relative liver weights were slightly increased at 1000 mg/kg bw/day. The only notable gross necropsy finding was a single animal at 1000 mg/kg bw/day with material deposited in the ureter and urinary bladder, along with multiple white areas in the kidneys (confirmed microscopically as urolithiasis with urothelial hyperplasia and hydronephrosis with pyelonephritis and transitional cell hyperplasia).

The pregnancy incidences and the mean numbers of corpora lutea and implantations were comparable across all test and control groups. Pre-implantation and post-implantation losses, live litter size, placental weight and foetal sex ratios were unaffected by treatment at all dose levels. At 1000 mg/kg bw/day, the incidence of engorged placentae was increased relative to the controls and above the relevant historical control maximum value, although the placental mean weight was identical to that of the controls. Foetal weights of both sexes at 1000 mg/kg bw/day were slightly, but statistically significantly, reduced.

There was a higher level (both foetal incidence and litter incidence) of microphthalmia in all the prothioconazole-exposed groups compared with the concurrent control. The incidences at 80 and 500 mg/kg

bw/day did not, however, show a dose-related increase and were within the relevant litter historical control data. The incidence of microphthalmia in the high-dose group was outside the historical control range and included two foetuses with bilateral microphthalmia (considered more likely to indicate an effect of treatment); in contrast, there were no bilateral incidences of this finding in any other group.

The full time-relevant historical control data for microphthalmia in this rat strain from the same laboratory are presented in Annex I. The litter incidences of microphthalmia at 80 and 500 mg/kg bw/d were below the incidences of five historical control studies, two of which were conducted one year either side of the prothioconazole study. Furthermore, the foetal incidence at 500 mg/kg bw/d was lower than the control incidence in six studies conducted between 1989 and 2002, one of these having been conducted in the same year as the prothioconazole study. The historical studies also often demonstrated a high inter-group variability within a given study, as illustrated in the table below (taken from the RAR). Overall, considering the absence of a dose-response relationship and incidences that were within the historical control ranges, the dossier submitter concludes that the cases of microphthalmia at 80 and 500 mg/kg bw/d were incidental and not related to prothioconazole exposure. However, this position and conclusion does not apply to the group that received the highest dose, 1000 mg/kg bw/d.

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

When the maternal toxicity data is grouped separately for those dams that produced pups with microphthalmia and for those that did not, it is shown that clear maternal toxicity was present in all dams at 1000 mg/kg bw/day, but that it was more pronounced in those dams that produced foetuses with microphthalmia (+MO) (see table below). Foetal weight (as an effect secondary to maternal toxicity) was reduced overall at this dose but the reduction was again more pronounced in those litters that included foetuses with microphthalmia. The severity of maternal toxicity therefore correlated positively with the degree of foetal toxicity (body-weight decrease) and with the occurrence of microphthalmia.

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

Group	**Maternal b.w. change (g)**		**Corrected mat. b.w. change d0-20 (g)**	**Feed intake d6-11 (g/animal/d)**	**Live foetal body weight (g)**
	d6-8	**d6-11**			
1000 mg/kg +MO	**-5.4** (-204% of Control)	**+4.3** (28% of Control)	**+21.1** (53% of Control)	**13.4** (72% of Control)	**3.38** (93% of Control)
1000 mg/kg -MO	**-1.4** (-127% of Control)	**+8.1** (52% of Control)	**+30.8** (77% of Control)	**16.3** (88% of Control)	**3.48** (96% of Control)
Control	**+5.2**	**+15.5**	**+40.0**	**18.6**	**3.63**

To further explore the hypothesis that the cases of microphthalmia in this study in the high-dose group might be related to maternal toxicity, the applicant for renewal under Regulation 844/2012 provided information on developmental toxicity studies in which rats were exposed to the substance cyfluthrin. Oral administration of cyfluthrin (up to a dose of 30 mg/kg bw/d, in a different rat strain (BAY:FB 30) than the one used in the first prothioconazole oral study) did not result in any embryotoxicity. This was a well-conducted study and justified

the conclusion that cyfluthrin did not exhibit developmental toxicity or teratogenicity. Rats of the same strain as the one used in the first prothioconazole oral study were exposed by inhalation to cyfluthrin at 2.55 mg/m^3 and above (equivalent to systemic doses of up to 3 mg/kg bw/d); these body burdens were substantially lower than the doses of up to 30 mg/kg bw/d given in the oral study. Cyfluthrin is a recognised respiratory irritant and in the inhalation developmental toxicity study clinical findings were apparent in the dams at levels of ≥ 2.55 mg/m^3 (bloody snout, ungroomed fur and piloerection); respiratory disturbances and hypoactivity were noted at 11.9 mg/m^3 and 12.8 mg/m^3 with supplementary oxygen, and a high-stepping gait and salivation at 11.9 mg/m^3 only. These findings were indicative of the anticipated respiratory irritation. At levels of 2.55 mg/m^3 and above the placental and foetal weights were reduced, and the foetuses exhibited an increased incidence of microphthalmia (foetal incidence = 0.41, 0.76, 0.41, 1.20, 5.44 % at 0 (air), 0 (vehicle), 0.46, 2.55, 11.9 mg/m^3) and retarded ossification. Oxygen supply (high-concentration group) resulted in reduction of maternal as well as developmental effects; in particular, the incidence of foetuses with microphthalmia was reduced to 2.91 %. Overall, the results indicated that the embryotoxic findings correlated with maternal toxicity (hypoxia with the resulting compensatory mechanisms of hypothermia and respiratory alkalosis, resulting in clinical signs of respiratory disturbances and hypoactivity). This was consistent with the increased incidence of microphthalmia in this strain of rat in the first prothioconazole oral study.

In the first rat study with prothioconazole, the only other notable visceral finding was dilatation of the renal pelvis at 1000 mg/kg bw/day, which was secondary to retarded foetal development at this dose, as indicated by decreased body weights and incomplete ossification (see below).

The only treatment-related skeletal findings were a dose-related increase in the variation rudimentary 14th rib and decreases in ossification. Toxicologically-relevant effects on ossification (distal and proximal phalanges, caudal vertebral bodies and 6th sternebral bone) were recorded in the high-dose group. Incomplete ossification is an indication of retarded foetal development and is consistent with the effects on foetal weights and renal pelvis dilatation at this dose. At all doses there was a dose-related increase in the incidence of rudimentary (punctiform and comma-shaped) supernumerary 14th lumbar ribs compared with the concurrent controls. The same finding was recorded in the range-finding study at 300 and 1000 mg/kg bw/d, but without a dose-response relationship. No fully-formed 14th ribs (considered a malformation) were observed in any dose group of either the range-finding or the main study. The historical control data (same laboratory and strain; Annex II) shows that rudimentary 14th ribs are a very common spontaneous variation in untreated rats and were reported in all but one of the 53 historical studies. However, this was not reflected in the concurrent control data for the present study; the control incidences for rudimentary 14th ribs were unusually low when compared with the historical data, being the lowest in the 52 studies. Foetal and litter incidences were within these historical control ranges (foetal = 0-24.4; litter = 0-57.1) up to and including 500 mg/kg bw/d (foetus = 10.6; litter = 54.5) and only slightly above at 1000 mg/kg bw/d. The dossier submitter concludes that in this study a treatment-related increase in this common variation occurred at 1000 mg/kg bw/d but not at the lower doses.

In further exploration of the microphthalmia in the first study, a second oral study was conducted in a rat strain (Wistar Hanover) with a negligible (essentially zero) background incidence of this finding (see Annex III). The sensitivity of this strain to specific developmental effects on the eye was shown in an oral study with the positive control substance all-trans-retinoic acid, in which gavage administration of 15 mg/kg bw/d during gestation days 6-15 resulted in cases of no eye bulge (foetal = 71.1 %, litter = 100 %), microphthalmia (foetal = 5.6; litter = 16.7) and anophthalmia (foetal = 22.2; litter = 41.7). None of these malformations occurred in the control group. The study with prothioconazole was designed with the advice of an external expert in developmental toxicity to provide objective measurements for the identification of microphthalmia, including the extraction, weighing and morphometric investigation of the eyes. The logic behind this study was that this strain of rats would show ocular developmental toxicity if it were a direct effect of prothioconazole, but would not be susceptible to foetal microphthalmia arising from physiological disturbance of the dams.

In a range-finding study in Wistar Hanover rats, the top dose of 1000 mg/kg bw/day resulted in excessive toxicity (three animals died of dehydration). Therefore, the highest dose administered in the main study was 750 mg/kg bw/day prothioconazole. In the main study there were no deaths or treatment-related clinical signs of toxicity at any dose. At 750 mg/kg bw/day net body weight gain during gestation was reduced by 13%. Overall body-weight gain was reduced by 46% and food consumption was decreased by up to 27% on gestation days 6-12. Consistent with previous studies, water consumption was increased on days 11-20 by up to 74%.

Indicators of functional impairments of kidneys and liver were observed at this dose, which correlated well with the established toxicological profile of prothioconazole in the rat (disturbed kidney function and resulting impaired systemic water homeostasis). There were no treatment-related findings in the low- and mid-dose groups.

There were no treatment-related reproductive effects, nor were there any significant differences in the litter size, the median percent male foetuses, or foetal or placental weights in any group tested.

Developmental investigations focussed on ocular and skeletal investigation in order to address the findings from the previous developmental toxicity study. No foetal visceral examinations were performed since the torso was evaluated for skeletal and cartilage development, with an emphasis on the occurrence of supernumerary rudimentary (punctiform and comma-shaped) ribs.

The ocular external examinations did not reveal a single foetus exhibiting microphthalmia in any dose group tested, including the 750 mg/kg bw/d dose group. No differences were observed on the individual or mean eye weights, eye-to-foetal weight ratios, or on eye measurements. The large sample size collected (control: 442 eyes; 750 mg/kg bw/d group: 482 eyes) demonstrated the normal distribution pattern of eye weight in the control and high-dose foetuses, with no indication for any individual values being outside the normal range of control animals. There was thus no evidence that prothioconazole caused microphthalmia in any dose group.

Skeletal evaluation revealed a possible treatment-related increase in the foetal incidence of supernumerary rudimentary (comma-shaped and punctiform; only comma-shaped statistically significantly increased) ribs at 750 mg/kg bw/d. The litter incidence was not statistically significantly affected for either type. A treatment-related effect on fully-formed supernumerary ribs was not discernible. The foetal incidence of the comma-shaped rudimentary ribs (21.2%) was only marginally outside the historical control range for the same laboratory and rat strain (maximum 18%), and that for punctiform ribs (33.6) was well within the upper boundary of the historical control range (maximum 52%, mean 32, median 29).

Overall, the oral studies in rats indicated that severely maternally toxic doses of prothioconazole increased the formation of microphthalmia and supernumerary ribs in strains of rat exhibiting high spontaneous incidences of these changes . Microphthalmia was not increased, however, in a rat strain that has a negligible spontaneous occurrence of this malformation, even when tested up to doses that were severely toxic to the dams.

Dermal administration of prothioconazole at doses up to 1000 mg/kg bw/d did not result in any developmental toxicity in rats.

10.10.4.2 Developmental toxicity in rabbits

In an oral range-finding study, there were treatment-related deaths of two females at 480 mg/kg bw/day (gestation days 23 and 26) and one female at 300 mg/kg bw/day (gestation day 24). Dose-dependent body-weight loss and persistently reduced food consumption were recorded in all dose groups. At 480 mg/kg bw/day, total post-implantation loss was recorded in one animal and foetal weights were reduced. There was no effect on other litter parameters. Nine of the 16 foetuses obtained from dams treated at 480 mg/kg bw/day were runts (small foetuses with bodyweights of 9.8-17.7 grams, compared with a mean in other groups of approximately 30 grams).

In the main developmental toxicity study, dose groups of 10, 30, 80 and 350 mg/kg bw/d were used. One animal of the 350 mg/kg bw/d group died on day 25 following reduced feed consumption and body-weight loss. There were no other treatment-related clinical signs. Food consumption and body-weight gain were statistically significantly reduced at 350 mg/kg bw/day (overall food consumption was 31% lower than controls, overall bodyweight gains were 47% of the controls at this dosage). Lower food consumption was most marked over days 6 to 19, and bodyweight loss was recorded on days 6 to 12.

Three females had abortions (days 22, 25 and 27) and three females had total litter resorption at 350 mg/kg bw/day, resulting in decreased overall litter size in this group, but not in dams which maintained live litters to day 28. Post-implantation losses were correspondingly higher at 350 mg/kg bw/day. Mean foetal weights were significantly reduced at 350 mg/kg bw/day (10-13% lower than controls) and mean placental weight was slightly (5.8%) lower. Pre-implantation loss, the incidence of dead foetuses and foetal sex ratio were unaffected

by treatment at 350 mg/kg bw/day. All reproductive parameters and foetal weights were unaffected by treatment at the other dose dose levels of up to 80 mg/kg bw/day.

The nature and incidences of external, visceral and skeletal abnormalities did not indicate an effect of treatment at any dose level.

In comparison with the oral gavage developmental study in the rat, it is notable that there was only a single incidence of microphthalmia in this study (in the low-dose group), and that was in a foetus with multiple malformations affecting the head. In the context of the overall pattern of findings in this study, this occurrence was clearly not related to prothioconazole treatment.

At 350 mg/kg bw/day there were some differences from the controls in the foetal incidences of incomplete and absent ossification of one or more sternebrae and phalanges of the digits, and of unossified 13th rib. However, in some cases the incidence was lower than controls and in others the incidence was higher than controls, indicating either advanced or retarded ossification in different structures. It is unlikely that such variability was the result of prothioconazole exposure.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

The developmental toxicity of prothioconazole has been extensively investigated in acceptable oral studies in rats and rabbits and in an acceptable dermal study in rats.

In the first oral study in Wistar rats (sub-strain Hsd Cpb:WU), a treatment-related increase in the incidence of foetuses and litters with microphthalmia was reported at the very high dose of 1000 mg/kg bw/d. Overt maternal toxicity, consistent with effects on kidney function and water / electrolyte homeostasis, was evident at this dose and also at the mid-dose level of 500 mg/kg bw/d. Effects on maternal body-weight were also apparent at these doses. An analysis of individual animal data showed that the dams with the lowest body-weight gains and feed intake were those that produced pups with microphthalmia; and that the lowest-weight foetuses (as a secondary effect to maternal toxicity) were in the affected litters. Considering this data and the high spontaneous occurrence of this malformation in this strain of rat, the applicant under Regulation 844/2012 concluded that the induction of microphthalmia was not a specific developmental effect of prothioconazole administration, but was rather an exacerbation of the background incidence as a result of maternal toxicity. To demonstrate the plausibility of this hypothesis, an inhalation study in the same strain with an irritant, non-teratogenic substance was submitted; the occurrence of microphthalmia at a higher incidence than that in the prothioconazole study, with partial abrogation of the effect upon oxygen supplementation (reduced hypoxia), established the non-specific nature of the finding.

Further corroboration was provided by a supplementary oral study in a strain of rat with a virtually zero background incidence of ocular malformations (Wistar Hanover, Crl:WI(HAN)). Even at doses up to 750 mg/kg bw/d, which were severely maternally toxic, no cases of microphthalmia or other ocular malformations were recorded. In contrast, a positive control substance tested in the same strain demonstrated the sensitivity of the system to specific developmental toxicants. Taking into account all the evidence, the dossier submitter concludes that prothioconazole did not directly and specifically induce malformations in rats, but as a result of maternal toxicity resulted in a secondary, non-specific increase in microphthalmia in a strain of rat with a relatively high spontaneous incidence of this malformation.

In both the rat studies, increased incidences of rudimentary 14th ribs were reported in the high-dose groups, although the increase at 750 mg/kg bw/d in the supplementary study was marginal. As shown by the historical control data for both rat strains, this is a very common variation (i.e, a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health[1]). It is also notable that in the range-finding experiment for the first study, the incidence of this variation in the mid-dose group was

[1] Chahoud I, Buschmann J, Clark R, Druga A, Falke H, Faqi A *et al*. (1999). Classification terms in developmental toxicology: need for harmonisation . Report of the Second Workshop on the Terminology in Developmental Toxicology Berlin, 27-28 August 1998. *Reprod. Toxicol.* **13**:77-82.

double that in the high-dose group, again demonstrating that this finding is common and often has a large inter-group variability. Furthermore, rudimentary supernumerary ribs (as opposed to full supernumerary ribs) in rats are generally regarded to be of low toxicological and biological relevance, since they do not persist beyond post-natal day 40 to 60 and do not appear to give a reliable prediction of hazard in human development[2345]. An increased incidence of rudimentary 14th ribs is also associated with maternal stress; in fact, it has been suggested that an increase in this variation could be used to indicate that a sufficient dose to induce some maternal toxicity has been administered in developmental toxicity studies, in the absence of more overt signs such as changes in maternal body-weight[2]. In the supplementary study, there was clearly not a treatment-related increase in supernumerary ribs at the low- and mid-dose levels of 20 and 80 mg/kg bw/d, respectively. In the first study, however, there was an apparent dose-related increase at all doses (80, 500 and 1000 mg/kg bw/d). The extensive historical control data showed that the statistical significance of the increases in the low- and mid-dose groups was confounded by an unusually low incidence in the concurrent control group. Furthermore, the incidences at 80 and 500 mg/kg bw/d were well within the historical control range.

In the first rat study, indicators of delayed development comprised reduced foetal weights, renal pelvis dilatation and delayed skeletal ossification only at 1000 mg/kg bw/d; the dossier submitter concludes that they were secondary to the severe maternal toxicity at this dose and thus were not indicators of specific developmental toxicity. Ossification changes were inconsistent in the oral rabbit study; the main treatment-related observation in this study comprised abortions, total litter losses and reduced foetal weights at the high-dose level of 350 mg/kg bw/d, which were secondary to the very severe maternal toxicity at this dose (death, body-weight loss, reduced body-weight gains, reduced food consumption). There was no indication of specific developmental toxicity in rabbits.

No developmental toxicity was recorded in a rat study via the dermal route when prothioconazole was tested at doses up to 1000 mg/kg bw/d.

The dossier submitter notes that the metabolite prothioconazole-desthio, the toxicology of which has been extensively investigated and which is more toxic in terms of qualitative and quantitative differences, is formed to only a very minor amount systemically (maximum 0.07 % in urine, maximum 0.45 % in bile; see section 9.1). Therefore, this metabolite is not expected to contribute to the reproductive toxicity of prothioconazole.

10.10.6 Comparison with the CLP criteria

There is no data on humans to inform on the developmental toxicity of prothioconazole, and so classification in category 1A is not appropriate.

Classification in category 1B is largely based on data from animal studies. Prothioconazole was not a developmental toxicant in rabbits when administered orally at a severely maternally toxic dose, nor in rats when administered dermally. Oral administration to rats resulted in increased incidences of two developmental effects, microphthalmia (malformation) and rudimentary supernumerary ribs (variation).

In an oral rat study conducted in a strain with a relatively high background incidence of microphthalmia, an increase in this malformation that was slightly above the historical control maximum was recorded at the high dose of 1000 mg/kg bw/d. This dose was associated with severe maternal toxicity that was consistent with the kidney effects and consequent renal dysfunction that characterised prothioconazole toxicity. Early body-

[2] Wickramaratne GA. (1988). The post-natal fate of supernumerary ribs in rat teratogenicity studies. *J. Appl. Toxicol.* **8**:91-94.

[3] Chernoff N, Rogers JM, Turner CI, Francis BM. (1991). Significance of supernumerary ribs in rodent developmental toxicity studies: postnatal persistence in rats and mice. *Fundam. Appl. Toxicol.* **17**(3):448-453.

[4] Mylchreest E, Harris SB (2013). Data interpretation: Using historical control data to understand supernumerary ribs, a common skeletal variation. In: Teratogenicity testing, methods and protocols, Barrow PC (editor), ISSN 1064-3745, ISBN 978-1-62703-130-1, Humana Press, Springer New York, Heidelberg, Dordrecht, London, 290-294.

[5] Ko EA, Park WE, Lim I, Yun J, Kim JH, Kang YK *et al.* (2010). Occurrence and fate of fetal lumbar rib induced by *Scutellariae radix* in rats. *Birth Defects Res.* **B 89**:201-206.

weight loss was followed by consistently decreased body-weight gains during gestation. These maternal effects also occurred, to a lesser extent, in the mid-dose group (500 mg/kg bw/d). Examination of individual animal data indicated that the severity of maternal toxicity correlated positively with the occurrence of microphthalmia in the high-dose group. However, in a rat strain with an essentially zero incidence of ocular malformations, no case of microphthalmia, anophthalmia, absent eye bulge or reduced eye weight was recorded at doses up to 750 mg/kg bw/d (the maximum tolerated dose in this strain) in a study with investigations specifically designed to detect ocular effects. The dossier submitter therefore concludes 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.

The same conclusion was reached for the increase in the common variation rudimentary supernumerary ribs that was observed in the first rat oral study, and marginally in the second rat oral study. As a variation that resolves post-natally and does not adversely affect survival or health, rudimentary supernumerary ribs are of less concern than malformations. It is also well recognised in the public literature that they are induced at maternally-toxic doses, as was the case in the present studies, with no apparent effect at doses that were not toxic to the dams.

Overall, therefore, prothioconazole resulted in developmental toxicity only at severely maternally-toxic doses: there was no evidence of an effect on development in the absence of maternal toxicity. For classification in category 1B, animal data shall provide clear evidence of an effect on development in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects. Microphthalmia was reported only in one rat study, with no cases in a second rat study that was specifically designed to identify this malformation. Furthermore, a higher level of the finding was only evident at the extremely high dose of 1000 mg/kg bw/d (severely maternally toxic) in the first study, with no increase at the next dose of 500 mg/kg bw/d (also maternally toxic), nor at 750 mg/kg bw/d in the second study. Likewise, the common variation supernumerary ribs was only increased at 1000 mg/kg bw/d in the first study and marginally at 750 mg/kg bw/d in the second study, a dose that resulted in overt maternal toxicity. The criterion for Category 1B that animal data '*shall provide clear evidence of an adverse effect…on development in the absence of other toxic effects*' is therefore evidently not met.

Some information on the nature of the developmental toxicity was provided by additional studies in rats. The occurrence of microphthalmia was increased when prothioconazole was administered at a very high dose to a strain of rat with a high spontaneous incidence of this malformation, but when administered at an equally high and maternally-toxic dose to a strain of rat with a negligible background incidence of this finding (and which was shown to be sensitive to chemicals with known ocular developmental toxicity), no cases of microphthalmia occurred. This raises substantial doubt over the direct involvement of prothioconazole in the induction of the malformation in the rat strain exhibiting a high spontaneous incidence of this malformations. With regards to supernumerary ribs, this variation had a relatively high spontaneous incidence in both strains of rat, and is a frequent observation in developmental toxicity studies or other situations where the dams are subjected to stress, as noted above. Overall, the available information has not established that prothioconazole induces developmental toxicity through a specific, direct effect. The dossier submitter thus concludes that prothioconazole does not meet the criteria for classification in category 1B.

Substances are classified in category 2 when there is some evidence from humans or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in category 1. Furthermore, the effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects. Following oral high-dose administration of the active substance to rats, increases in microphthalmia (one, rat strain exhibiting a high spontaneous incidence of this malformation) and supernumerary ribs (both rat strains) occurred; therefore, oral administration of prothioconazole was associated with developmental toxicity. On this basis, a case for classification in category 2 could be made. However, these developmental effects were only reported at extremely high doses (1000 mg/kg bw/d for both findings in the first rat study; marginal change in the incidence of supernumerary ribs at 750 mg/kg bw/d in the second study); neither finding was increased at the next dose of 500 mg/kg bw/d (in the first study), at which maternal toxicity was still evident but less severe. Rudimentary supernumerary

ribs are a very common variation that have no effect on survival and do not persist post-natally; as such, an increase in this finding at doses that also cause maternal toxicity does not support classification. Prothioconazole's involvement in the induction of microphthalmia in rats is not clear: there is no known mode of action for this substance by which this effect might have been expressed, and the finding was only reported in one study and when administered at a very high dose. The most likely explanation for both findings was that prothioconazole was not directly, specifically responsible; rather, the systemic effects suffered by the dams, with consequences on the growth and development of the foetuses, resulted in disruptions to normal development and an increase in spontaneous findings. This supposition is supported by the absence of any cases of microphthalmia in the second rat study. No treatment-related malformations or variations were reported in an oral rabbit study at doses that were excessively toxic.

The findings in the two developmental toxicity studies in rats with prothioconazole could support no classification for developmental toxicity, or classification in category 2. The main considerations are the uncertainty around the direct causative involvement of this substance in the occurrence of microphthalmia in only one of the studies; the extremely high dose at which this occurred; and the nature and reversibility of the supernumerary ribs (a common variation) in association with maternal toxicity. On balance, taking into account all the available evidence, the dossier submitter concludes that the criteria for classification in category 2 are not met and proposes not to classify prothioconazole for adverse effects on development of the offspring.

10.10.7 Adverse effects on or via lactation

The potential of prothioconazole to elicit adverse effects on or via lactation has been investigated in a two-generation study in rats (see section 10.10.1).

In this study, parental general systemic toxicity was observed at the intermediate- (100 mg/kg bw/d) and high-dose (750 mg/kg bw/d) levels. Effects at the intermediate dose (100 mg/kg bw/d) included lower body-weight gains, decreased thymus weights and increased liver weights. At the high dose of 750 mg/kg bw/day, similar but more marked effects were recorded, along with reduced efficiency of food utilisation, increased kidney weights and histopathological findings in the liver and kidneys consistent with effects seen in previous repeated-dose toxicity studies in rats (hepatocytomegaly, multifocal chronic nephrosis). There were also clinical indications of disruption of the normal kidney function and water / electrolyte homeostasis (urine stains, dehydration). The parental toxicity at 750 mg/kg bw/d was therefore considered to be severe.

Even with this marked maternal toxicity, post-natal survival of pups was not affected by prothioconazole exposure: there were no effects on lactation or viability indices in either generation. There were effects on development of the pups at the high-dose level. Pup weight-gain was significantly reduced during lactation (up to 27 % in F1 pups (day 4-7); up to 18 % in F2 pups (day 4-7 and 7-14). The mean pup weight (males and females combined) was reduced in F1 pups from day 4 on (10-17 %) and in F2-pups from day 7 on (6-12 %) compared with controls. The maximum reduction in both generations (17 and 12 % respectively) was observed on day 14 but partially recovered up to day 21 (14 and 8 % respectively), when pups had started to eat solid food in addition to the dams' milk. Up to day 44 (day 22 post-weaning) body weights further recovered (only 10 % lower than control).

Dehydration at doses of ≥ 500 mg/kg bw/d in rats in several repeated-dose studies was severe and even resulted in the deaths of some dams in a range-finding developmental toxicity study and drastically increased water consumption in the main developmental toxicity study, both conducted in the same rat strain as the two-generation study. It could therefore be postulated that the lactating dams suffered from dehydration, which impacted milk production and thus the 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.

10.10.8 Comparison with the CLP criteria

Substances that are absorbed by women and have been shown to interfere with lactation shall be classified and labelled to indicate this property hazardous to breastfed babies. Effects in the mother can adversely impact the breast milk (either in terms of the quantitiy produced or the quality of the milk produced). However, if a

substance causes overt toxicity in the mother, this may indirectly impair milk production or impair maternal care as a non-specific secondary effect and should not lead to classification.

No data from humans are available.

The maternal toxicity at 750 mg/kg bw/day observed in the two-generation rat study (primarily related to kidney dysfunction and disturbed water homoeostasis/dehydration) is considered to be very strong and possibly resulted in reduced milk production as a result of dehydration. Therefore, the dossier submitter considers the observed effect on pup body-weight (gain) during lactation to be a non-specific secondary effect caused by the overt toxicity in the mothers. No classification for reproductive toxicity concerning effects on or via lactation is proposed.

10.10.9 Conclusion on classification and labelling for reproductive toxicity

Not classified – Conclusive but not sufficient for classification

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.

Table. *Relevant reproductive parameters of F0- and F1-generation animals.*

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. 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. 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

[a] Historical control range from 7 studies in Wistar rats performed 1998-2001. Letters in bold were considered treatment-related by the DS.

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.

Additional key elements

In the public consultation, one report (Bayer, 2018) was submitted as part of the comments from the industry association. The report called "prothioconazole is chemically not a triazole, it is a triazolinethione, and thus does not cause the developmental and reproductive toxicity observed for several classical triazoles", provided evidence that prothioconazole is a triazolinethione and exhibits different physicochemical and toxicological properties from triazoles, such as different pattern and affinity for binding to CYP450 enzymes.

RAC agrees that prothioconazole is structurally a triazolinethione and not a triazole. Data from the scientific literature included in the report show that prothioconazole had a lower affinity to target CYP450s, such as CYP51 and CYP19, as compared to a number of azoles/triazoles. The report also provided computational chemistry data to illustrate/support this. In several bioassays, prothioconazole had a lower potency than azoles in terms of receptor binding and toxicity *in vitro*. RAC acknowledges that the pattern of developmental toxicity of prothioconazole is different from triazoles in terms of e.g. late foetal mortality and craniofacial malformations such as cleft palate.

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

Table. *Summary of skeletal/cartilaginous findings in rats after prothioconazole exposure (% foetal (litter) incidences, RAR Tables B.6.6.14 and 6.6.20, modified by RAC).*

Parameter	**prothioconazole (mg/kg bw/d)**				
	0	**80**	**500**	**1000**	**HCD [c]**
Mean foetal weights combined (g)	3.63	3.57	3.57	3.45**	3.60 – 3.84
Skeletal Examination					
No. foetuses (litters) evaluated	152 (26)	155 (26)	142 (22)	147 (24)	
Wavy ribs (variant)	15.1 (46.2)	6.5* (38.5)	4.2** (18.2)	2.0** (12.5)*	0.8-9.4 (4.3-30.0)
Rudimentary 14th rib (variation)	0.7 (3.8)	7.1* (42.3)**	10.6* (54.5)**	25.2** (62.5)**	0.0-24.4 [d] (0.0-57.1)
Distal phalanges – Digit(s)					
5th digit, right – unossified	2.6 (15.4)	5.2 (23.1)	4.9 (27.3)	10.2* (37.5)	0.0-69.9 (0.0-100.0)

5th digit, left – unossified	3.9 (19.2)	10.4 (30.8)	9.9 (27.3)	14.3** (45.8)	0.0-70.8 (0.0-100.0)
Proximal phalanges – Digit(s)					
3rd digit, right – unossified	70.4 (96.2)	78.1 (92.3)	73.9 (95.5)	86.4** (100.0)	43.1-80.5 (70.8-100.0)
3rd digit, left – unossified	76.3 (100.0)	81.3 (96.2)	79.6 (100.0)	92.5** (100.0)	51.2-85.0 (72.2-100.0)
4th digit, right – unossified	74.3 (96.2)	81.3 (96.2)	79.6 (95.5)	90.5** (100.0)	46.3-83.2 (79.2-100.0)
4th digit, left – unossified	82.2 (100.0)	85.8 (100.0)	82.4 (95.5)	95.9** (100.0)	56.1-88.5 (75.0-100.0)
Distal phalanges – Toe(s)					
1st toe, right – unossified	7.5 (26.9)	5.5 (23.1)	4.3 (27.3)	19.3* (66.7*)	0.0-57.5 (0.0-100.0)
4th toe, right – incompletely ossified	28.5 (69.2)	38.3 (73.1)	26.8 (77.3)	36.1 (100.0*)	10.7-76.8 (39.1-100.0)
4th toe, left – unossified	7.3 (23.1)	9.7 (46.2)	4.2 (22.7)	17.0* (50.0)	1.3-48.7 (7.7-77.3)
5th toe, right – unossified	12.0 (34.6)	20.6 (69.2)	17.6 (54.5)	33.3** (83.3**)	2.3-77.9 (8.7-100.0)
5th toe, left – unossified	14.6 (42.3)	21.9 (65.4)	15.6 (50.0)	31.3** (79.2*)	1.3-80.5 (7.7-100.0)
Sternebra(e)					
6th sternebra – incompletely ossified	16.4 (42.3)	18.1 (46.2)	19.0 (45.5)	36.7** (79.2)	2.4-22.1 (12.5-59.1)
Caudal vertebral body(ies)					
4th caudal vertebral body – present	100.0 (100)	96.1 (100)	96.5 (100)	89.8** (100)	92.0-98.7 (100.0)
5th caudal vertebral body – present	48.7 (92.3)	42.6 (84.6)	26.1** (72.7)	17.7** (58.3*)	39.8-78.0 (80.0-100.0)

* $p < 0.05$; ** $p < 0.01$ (Fisher's exact test); [a] unilateral; [b] bilateral, [c] Historical control data range 1992-94 (9 studies with 196 litters and 2149 foetuses), [d] Historical control data range 1991-2001 (40 studies)

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.

Table. *Summary of notable skeletal variant findings in rabbits after prothioconazole exposure (RAR Table B.6.6.27).*

Parameter	Incidence (%) in groups treated at (mg/kg bw/day):				
	0	10	30	80	350
No. foetuses evaluated	176	210	190	205	152
Unossified sternebrae 5	13	12	16	18	20*
Unossified 13th rib	49	63**	65**	54	37*
Left forelimb:					
- incomplete ossification metacarpal 1	34	31	28	27	0**
- incomplete ossification digit 5 phalanx	56	58	64	48	28**
- unossified digit 5 phalanx	43	41	54	52*	72**
Right forelimb:					
- incomplete ossification metacarpal 1	34	31	28	28	0**
- incomplete ossification digit 5 phalanx	53	57	61	44*	27**
- unossified digit 5 phalanx	46	42	38	55*	72**
Left hind limb:					
- incomplete ossification digit 4 phalanx	59	52	52	51	26**

- unossified digit 4 phalanx	14	17	12	22*	26**
Right hind limb: - incomplete ossification digit 4 phalanx - unossified digit 4 phalanx	 59 14	 50 19	 51 13	 50 23*	 26** 26**
No. litters examined	20	24	21	23	17
Left forelimb: - incomplete ossification metacarpal 1 - incomplete ossification digit 5 phalanx	 95 100	 67* 100	 95 100	 61** 96	 0** 76*
Right forelimb: - incomplete ossification metacarpal 1	 95	 67*	 90	 61**	 0*
Left hind limb: - incomplete ossification digit 4 phalanx	 100	 96	 95	 96	 76*
Right hind limb: - incomplete ossification digit 4 phalanx	 100	 96	 95	 96	 76*

* p<0.05, ** p<0.01 (Fisher's Exact Test)

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**.

10.11 Specific target organ toxicity – Single exposure

The acute studies that are relevant for the assessment of the specific target organ toxicity of prothioconazole after single exposure are reported in sections 10.1 to 10.3. An acute neurotoxicity study is also available and is summarised below.

Table 35: Summary table of animal studies on STOT SE

Study, species, test substance, purity	Doses	Main effects
Acute neurotoxicity Oral (gavage) OECD 424 (1997) GLP Rat, Wistar, Crl:WI(HAN)BR Males & Females 12/sex/group Prothioconazole (purity 97.6 - 98.8 %) Vehicle: aqueous 0.5 % methylcellulose/ 0.4 % Tween 80 Anonymous (2000b)	0, 200, 750 and 2000 mg/kg bw Actual doses: 0, 218, 847 and 2240 mg/kg bw Single dose	There were no deaths <u>2000 mg/kg bw</u> Perianal brown staining (resolved within 5 days), soft faeces (4 hrs post-treatment only) ↓ motor activity, ↓ locomotive activity (both sexes 4 hours post-treatment only) <u>750 mg/kg bw</u> Perianal brown staining (resolved within 5 days), soft faeces (4 hrs post-treatment only) ↓ motor activity, ↓ locomotive activity (males 4 hours post-treatment only) <u>200 mg/kg bw</u> No treatment-related effects

↑ / ↓ = increased/decreased compared with control. Unless otherwise stated, effects were seen in both sexes.

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Four studies were available to assess the specific target organ toxicity of prothioconazole upon single exposure.

In an acute neurotoxicity study, there were no deaths or effects on body weights. The only notable functional observation battery (FOB) signs were perianal staining in both sexes at 750 and 2000 mg/kg bw and a transient effect on motor activity and locomotor activity which was reduced in both sexes at 2000 mg/kg bw and in males at 750 mg/kg bw/day; in both cases the effect was observed at 4 hours post-treatment only. There were no FOB effects at 7 or 14 days post-treatment. Microscopic examination of tissues at 2000 mg/kg bw did not reveal any effect of treatment. There were no gross necropsy findings and no effects on brain weight, nor were there any neurohistopathological changes in nerve tissue or persistent signs of neurobehavioral toxicity.

In an acute oral toxicity study (see section 10.1), the only clinical signs were decreased motility and diarrhoea at the only dose tested of 6200 mg/kg bw.

In an acute dermal toxicity study (see section 10.2), animals showed partial reddening of the skin at the limit dose tested of 2000 mg/kg bw.

In an acute inhalation toxicity study (see section 10.3), at the single concentration tested of 4.99 mg//l/4hr, clinical signs on the day of exposure consisted of decreased rectal temperature, piloerection, absent grooming, bradypnea, laboured breathing, nasal discharge, red encrustation around the muzzle and laboured breathing. All of these effects had resolved within three days of exposure. There were no findings at necropsy and the decreased temperature and laboured breathing were considered to be related to sensory irritation caused by dust exposure.

No human data are available.

10.11.2 Comparison with the CLP criteria

Classification into STOT-SE category 1 or 2 might be appropriate if a substance is presumed to produce significant and/or severe target organ toxicity in humans following single exposure, on the basis of observations in humans or evidence from animal studies or is presumed to have the potential to cause harm to human health following single exposure.

In the acute toxicity studies with prothioconazole, there was no evidence of specific target-organ toxicity after a single exposure. Moreover, the doses tested generally exceeded the guidance cut-off values for category 2 (≤ 2000 mg/kg bw for oral and dermal; ≤ 5 mg/l/4hr for inhalation). Classification of prothioconazole for STOT-SE category 1 or 2 is not warranted.

At present category 3 classification for STOT-SE refers to transient target organ effects and is reserved for narcotic effects and respiratory tract irritation. Decreased motor and locomotor activities were observed in the acute neurotoxicity study at four hours post-treatment only and had resolved thereafter. These are typical mild indications of the animals feeling slightly unwell immediately after dosing and do not constitute a specific neuro-pharmaco-toxicological narcotic effect. Therefore, classification of prothioconazole in category 3 STOT-SE for narcotic effects is not appropriate.

Regarding a possible STOT-SE Category 3 classification for respiratory tract irritation, the observed laboured breathing, serous nasal discharge and red encrustation around the muzzle/nostrils (all reversible within three days of exposure) that were observed in the acute inhalation study could indicate respiratory tract irritation. However, at necropsy no histopathological findings were observed that would meet the criteria for classification as described in Annex 1 3.8.2.2.1 of Regulation (EC) 1272/2008. The change in breathing rate and decreased body temperature were attributed to a non-specific response to sensory irritation from exposure to dust, and, thus, not to a specific irritative potential of prothioconazole. Since there was no evidence of specific respiratory tract irritation, it is proposed not to classify prothioconazole for STOT-SE category 3.

10.11.3 Conclusion on classification and labelling for STOT SE

Not classified – Conclusive but not sufficient for classification

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**.

10.12 Specific target-organ toxicity - repeated exposure

The specific target-organ toxicity of prothioconazole upon short-term repeated exposure has been investigated in 28-day and 90-day studies in rats, a 90-day study in mice and 90-day and one-year studies in dogs. A 28-day dermal toxicity study in rats is also available. Chronic/carcinogenicity studies in rats and mice are described in detail in section 10.9 and briefly summarised below.

Table 36: Summary table of short-term repeated-dose animal studies

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP Guideline value for classification (mg/kg bw/d)	Results
RATS				

Method, guideline, deviation(s) from the guideline (if any)	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**CLP Guideline value for classification (mg/kg bw/d)**	**Results**
Sub-acute 28-day oral (dietary) OECD 407 (1995) Deviations: no FOB or motor activity assessments GLP Vehicle: peanut oil Prothioconazole purity: 99.5 % Anonymous, 1997c	Rat, Wistar HsdCpb:WU 5/sex/group	0, 196, 1480, 9250 ppm Equivalent to: Males: 18.6, 146 and 952 mg/kg bw/d Females: 18.8, 151 and 1033 mg/kg bw/d 28 days	Cat 1 = 30 Cat 2 = 300	No deaths or overt clinical signs of toxicity <u>9250 ppm (952 and 1033 mg/kg bw/d in males and females respectively)</u> ↓ Body-weight gain in males only (↓ by 22% week 4), ↑ water consumption (36% in males and 47% in females), ↑ food consumption ↑ ALAT, ALP, cholesterol and urea in males and females, ↓ T4, ↑TSH & ↑ calcium in females ↓ urinary volume, ↑ urine density (males & females) ↑ liver weights in females (relative by 23 %), pale marbled kidneys with lesions in males and females, basophilic tubules and cortical tubular dilatation more frequent and severe in males, ↑ cell proliferation in kidneys <u>1480ppm (146 and 151 mg/kg bw/d in males and females respectively)</u> ↑ ALAT and ALP in males and females <u>196ppm (18.6 and 18.8 mg/kg bw/d in males and females respectively)</u> No treatment-related findings

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP Guideline value for classification (mg/kg bw/d)	Results
Sub-acute 28-day oral (dietary & gavage comparison study) Guideline 407 (1995) Deviations: full investigations not carried out (comparison study) GLP Vehicle: silica gel & peanut oil (diet), 0.5% aqeous carboxymethyl-cellulose (gavage) Prothioconazole purity: 99.5 % Anonymous, 1998c	Rat, Wistar, HsdCpb:WU 5/sex/group	Diet 0 ppm (control) 10,000 ppm prothioconazole (equivalent to 1036 – 1066 mg/kg bw/d) 10,000 ppm silica stabilised prothioconazole (equivalent to 1034 – 1082 mg/kg bw/d) Gavage 0 or 1000 mg/kg bw/d prothioconazole in 0.5% aqueous carboxymethyl-cellulose	Cat 1 = 30 Cat 2 = 300	No deaths 10 000 ppm prothioconazole (diet) Piloerection ↓ body –weight gain ↑ food consumption ↑ water consumption ↑ absolute & relative liver weights (females) ↑ bilaterally occurring basophilic tubules Cytoplasmic change in centrilobular hepatocytes 10 000 ppm silica stabilised (diet) prothioconazole ↓ body –weight gain 1000 mg/kg bw/d (gavage) Piloerection ↓ body –weight gain ↑ food consumption (water consumption not measured in this group) ↑ ALT (both sexes), ↑ ALP (females), ↑ urea (males) ↑ absolute & relative liver weights (16 & 17 %, females only) ↓ absolute & relative kidney weights (13 & 8%, males only) ↑ bilaterally occurring basophilic renal tubules Cytoplasmic change in centrilobular hepatocytes
Sub chronic 90-day oral (gavage) OECD 408 (1998) Deviations: no FOB or motor activity assessments GLP Vehicle: 0.5 % aqueous Tylose Prothioconazole purity 97.6% Anonymous, 1999e	Rat, Wistar, HsdCpb:WU 10/sex/group Satellite group of 5/sex for 4 weeks for immune-toxicity investigations	0, 20, 100, 500 mg/kg bw/d Once daily for 14 weeks 4 week recovery period: control and high dose	Cat 1 = 10 Cat 2 = 100	5 deaths unrelated to toxicity: 3 deaths from blood collection and 2 from mis-dosing 500 mg/kg bw/day 1/10 females killed in moribund state, with necropsy findings in the kidney ↑ water consumption (by 20-24% males and females) ↓ ASAT, ↑ cholesterol, ↓ triglycerides, ↓ urine volume (experimental artefact resulting from insufficient water supply) ↑ liver weights (9% relative in females), ↓ spleen weights (13% relative in males) Hepatocyte hypertrophy (graded as slight) & cytoplasmic change in males (6/10; none in other groups) and females (2/10; none in other groups) Basophilic tubules in the kidneys (males only,

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP Guideline value for classification (mg/kg bw/d)	Results
				incidence = 5/10 (minimal), 8/10 (minimal), 8/10 (minimal), 9/10 (minimal / slight) at 0, 20, 100, 500 mg/kg bw/d). 100 mg/kg bw/day No treatment-related findings 20 mg/kg bw/day No treatment-related findings
90-day neurotoxicity study (gavage) OECD 424 (1997) Deviations: no investigations during weeks 1 or 2 GLP Prothioconazole purity > 97.6 % Vehicle: aqueous 0.5 % methyl-cellulose/ 0.4 % Tween 80 Anonymous, 2001g	Rat, Wistar, Crl:WI(HAN)BR 12/sex/group	0, 100, 500, 1000 mg/kg bw/day Analytically determined actual doses: 0, 98, 505 and 1030 mg/kg bw/day Gavage, 5 days/week for 13 weeks	Cat 1 = 10 Cat 2 = 100	No deaths 1000 mg/kg bw/day ↓ body-weight gain (8.4% males) FOB effects: urine staining (slight/moderate to severe), oral staining, (3/12 males & 1/12 females), ↓ motor activity (males), ↓ locomotor activity (males & females) ↑ wetness/staining of ventrum No neurohistopathological changes in nervous tissue 500 mg/kg bw/day FOB effects: urine staining (slight/moderate to severe) ↑ wetness/staining of ventrum No neurohistopathological changes in nervous tissue 100 mg/kg bw/day No treatment-related effects
MICE				
Sub-chronic 90-day oral (gavage) OECD 408 (1998) Deviations: no FOB or motor activity assessments GLP Vehicle: 0.5 % aqueous tylose Prothioconazole purity 97.6 % Anonymous,	Mouse, CD-1 Crl:CD-1(ICR)BR 10/sex/group	0, 25, 100, 400 mg/kg bw/day Once daily for 14 weeks	Cat 1 = 10 Cat 2 = 100	No treatment-related deaths (6 deaths across all groups from mis-dosing & during blood collection) 400 mg/kg bw/day ↑ cholesterol (females), ↓ bilirubin (males and females), ↓ protein and albumin (males), ↑ hepatic enzyme activity ↑ liver weight (relative by 56% and 37% in males and females, respectively), enlarged liver (3/10 males & 1/10 females), liver lobulation (5/10 males) Hepatocellular hypertrophy (9/10 males and 10/10 females), centrilobular fatty change (10/10 males, but also in 8/10 controls),

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP Guideline value for classification (mg/kg bw/d)	Results
1999f				periportal fatty change (6/10 females) <u>100 mg/kg bw/day</br> ↑ liver weight (relative by 21% in males and 15% in females) Liver lobulation (1/10 males), hepatocellular hypertrophy (9/10 males and 3/10 females), centrilobular fatty change (10/10 males, but also in 8/10 controls) <u>25 mg/kg bw/day</u> No adverse effects
DOGS				
Sub-chronic 90-day oral (gavage) OECD 409 (1998) GLP Vehicle: aqueous 0.5 % methyl-cellulose/ 0.4 % Tween 80 Prothioconazole purity > 98.1 % Anonymous, 2001h	Dog, Beagle 4/sex/group	0, 25, 100, 300 mg/kg bw/day 5 days/week for 13 weeks 8 week recovery period (control and high-dose groups)	Cat 1 = 10 Cat 2 = 100	1 death from misdosing (high-dose recovery-group female). No treatment-related clinical signs <u>300 mg/kg bw/day</u> ↑ ALT (males and females), ↑ ALP (males and females), ↑ GGT (females), ↓ T4 (mainly females) Cysts on kidneys (1/4 main-group males and 2/4 recovery males) ↑ liver, kidney and thymus weights (female), ↑ liver and kidney weights (males) Histopathological findings in kidneys (described in text) <u>100 mg/kg bw/day</u> ↑ ALT and ALP (males and females not statistically significant), ↑ hepatic microsomal enzymes (2 fold) ↑ liver weights (11% in females but not statistically significant; no change in males) Histopathological findings in kidneys (described in text) <u>25 mg/kg bw/day</u> No adverse effects
Sub-chronic one-year oral toxicity (gavage) OECD 452 (1981) GLP Vehicle: aqueous 0.5 % methyl-	Dog, Beagle 4/sex/group	0, 5, 40, 125 mg/kg bw/day Gavage, 5 days/week for 52 weeks	Cat 1 = 2.5 Cat 2 = 25	No deaths and no dose-related clinical signs. <u>125 mg/kg bw/d</u> ↓ Body-weight gain (by 14 % in males & 42 % in females) ↑ Serum ALP (females) ↑ Liver weight (relative by 23 % in males, 35 % in females) & kidney weight (relative by

Method, guideline, deviation(s) from the guideline (if any)	Species, strain, sex, no/group	Dose levels, duration of exposure	CLP Guideline value for classification (mg/kg bw/d)	Results
cellulose/ 0.4% Tween 80 Prothioconazole (purity > 98.4 %) Anonymous, 2001i				31 % in females; no change in absolute weight) Histopathology: liver (pigmentation); kidneys (chronic inflammation, crystalline material in tubules, pigmentation; see text) <u>40 mg/kg bw/d</u> ↓ Body-weight gain in males (↓ by 11 %) ↑ Serum ALP (females) Histopathology: kidneys (chronic inflammation, pigmentation; see text) <u>5 mg/kg bw/d</u> No adverse effects
Subacute 28 day dermal OECD 410 (1981) GLP Vehicle: water Prothioconazole purity 98.5 % Anonymous, 2000c	Rats, Wistar 10/sex/group	0, 100, 300 and 1000 mg/kg bw/day 6 h/day for 4 weeks: 5 days/week for 3 weeks, 7 days/week for the final week	Cat 1 =60 Cat 2 = 600	No adverse effects at any dose

↑ / ↓ = increased/decreased compared with control.

10.12.1 Oral

<u>Rats</u>

The short-term repeated-dose toxicity of prothioconazole in rats has been investigated in 28-day and 90-day studies. Dietary stability of prothioconazole has been an issue in studying its repeated-dose toxicity.

An initial 28-day study indicated that prothioconazole was relatively unstable when formulated in the diet. In a second 28-day study, which was performed to compare the effect of different oral administration methods, the lowest plasma concentrations were obtained with silica-stabilised diet formulation; neat diet formulation resulted in plasma concentrations approximately 2-fold higher, whilst gavage dosing produced the highest plasma concentrations (approximately 6-fold higher), which was consistent with the more marked effects seen in gavage-dosed animals compared with diet-treated animals.

Hence, gavage dosing was used for all the subsequent repeated-dose studies in rats, mice and dogs. Gavage dosing of rats for 28 days at 1000 mg/kg bw/d (the only dose tested in this second study) resulted in some clinical signs, decreased body-weight gain, increased clinical-chemistry changes, changes in liver and kidney weights and some renal histopathology findings. At equivalent doses in the first 28-day study, similar effects were reported, whilst the only finding at the next dose of 146 / 151 mg/kg bw/d was a slight change in some liver-enzyme activities that the dossier submitter concludes were not adverse. There were no treatment-related findings at the lowest dose of 18.6 / 18.8 mg/kg bw/d.

In a 90-day gavage study, prothioconazole was administered to Wistar rats at doses of 0, 20, 100 and 500 mg/kg bw/d; additional recovery groups (control and high-dose) were maintained for four weeks after withdrawal of

exposure. One female in the high-dose group was sacrificed in a moribund state. Necropsy of this animal revealed dilations in the urinary bladder, inflammation of the tongue and the presence of basophilic tubules in the kidneys. Water consumption was increased by 20-24% at this dose compared with controls, whilst urinary output was decreased with a corresponding increase in urine density and protein concentration; the urinary effects were considered by the study authors to be experimental artefacts that resulted from insufficient water supply during the over-night period. Biochemistry measurements revealed changes in some parameters that were consistent with liver effects. Liver weights were increased (only in females and by < 10 %) and hepatocyte hypertrophy and cytoplasmic changes were recorded during histopathology, consistent with adaptive liver changes. Spleen weights were reduced in males but without histopathological correlates. Kidney weights were unaffected in this study, but there was an increase in the incidence and prominence of basophilic tubules (9/10 males, 4 of minimal severity and 4 of slight severity, compared with 5/10 in the controls, 4 minimal and 1 slight; no change in females). Therefore, the kidney was the main target organ in this study, with males being more affected than females. No effects were seen at 20 and 100 mg/kg bw/day. In the recovery-group animals, no organ weight changes or histopathology findings were observed.

In a 90-day neurotoxicity study in rats, there were no indications of neurotoxicity at doses up to 1000 mg/kg bw/d. The only treatment-related clinical sign was urine staining at ≥ 500 mg/kg bw/day, which correlated with the only notable necropsy finding: increased incidence of wetness and/or staining of the ventrum at 500 and 1000 mg/kg bw/d. There were no effects at the lowest tested dose, 100 mg/kg bw/d.

Mice

Mice received oral (gavage) administration of prothioconazole for 90 days at doses of 0, 25, 100 and 400 mg/kg bw/d. There were no effects on body weights or haematology parameters at any dose. The six deaths during the study were attributed to mis-dosing and blood collection errors. The only biochemical changes observed were in the high-dose group (400 mg/kg bw/day) and consisted of an increase in cholesterol (females), a decrease in total protein and albumin (males) and a decrease in bilirubin (both sexes). Liver-weight increases (adverse at both doses) and liver lobulation were noted at 400 and 100 mg/kg bw/day (see table below). Additionally, an enlarged liver was observed in 3/10 males and 1/10 females of the high-dose group. Associated histopathology findings were observed at 100 and 400 mg/kg bw/d, consisting of hepatocellular hypertrophy and centrilobular fatty change (males, although also observed in 8/10 control animals). A higher incidence of centrilobular fatty change was observed in females at 100 mg/kg bw/d (5/10) than in controls, but since the number of affected animals was lower in the high-dose group (2/10), the dossier submitter does not consider this to be clear evidence of a treatment-related effect. The incidence of periportal fatty change was, however, increased in the high-dose females. There were no adverse effects at 25 mg/kg bw/day. The liver was the target organ in this mouse study; unlike in the rat studies, toxicity to the kidneys was not evident.

Summary of liver findings in 90-day mouse study

Parameter	No. animals affected (mean severity) in:							
	Males treated at (mg/kg bw/day):				Females treated at (mg/kg bw/day):			
	0	25	100	400	0	25	100	400
Gross lesions: - no. examined - overt liver lobulation - enlarged liver	 10 0 0	 10 0 0	 10 1 0	 10 6[a] 3	 10 0 0	 10 0 0	 10 0 0	 10 0 1
Organ weights (% control)[b]: - liver (absolute) - liver (relative)	 - -	 108% 117%	 113% 121%	 144% 156%	 - -	 102% 108%	 114% 115%	 139% 137%

Liver (no. examined)	10	10	10	10	10	10	10	10
- cytoplasmic change	0	0	9 (1.6)	9 (2.3)	0	0	3 (1.0)	10 (1.4)
- hypertrophy	0	1 (1.0)	9 (1.9)	9 (2.7)	0	0	3 (1.0)	10 (1.4)
- vacuolation	1 (1.0)	0	1 (3.0)	6 (2.0)	0	0	0	1 (2.0)
- focal necrosis	0	0	0	3	1	0	0	2
- fatty change								
-- centrilobular	8 (1.0)	5 (1.0)	10 (1.6)	10 (2.6)	0	3 (1.0)	5 (1.0)	2 (1.5)
-- periportal	0	0	0	0	1 (1.0)	1 (1.0)	0	6 (1.5)

Severity scores: 1 = minimal; 2 = slight; 3 = moderate

[a] includes one male that died due to blood sampling procedure

Dogs

The short-term repeated-dose toxicity of prothioconazole in dogs has been investigated in 90-day and one-year oral (gavage) studies.

In the 90-day study, prothioconazole was administered at doses of 0, 25, 100 and 300 mg/kg bw/d; recovery groups of additional control and high-dose animals were included. There were no treatment-related deaths or clinical signs of toxicity, and body-weight development was not clearly or consistently affected. Increases in ALT and ALP were observed in both sexes at 300 mg/kg bw/d, which in females showed only partial recovery, with slight increases also recorded at 100 mg/kg bw/day. Increases in the weights of the liver and kidneys in both sexes and the thymus in females in the high-dose group were observed. A smaller liver weight increase (11% of controls) was also observed at 100 mg/kg bw/day in females only. None of the organ weight changes persisted to the end of the recovery period.

Treatment-related histopathological findings were recorded in the kidneys of both sexes at 100 and 300 mg/kg bw/day. Males were affected to a greater extent than females. Rather general terms, such as inflammation, chronic, were used in the study report summary table (reproduced below), but the study authors described the changes as follows. *'The findings that indicated chronic inflammation consisted of multifocal chronic interstitial fibrosis in the cortex (often extending into the medulla), minimal inflammatory cell infiltrates, particularly lymphocytes in most of the lesions and compensatory hyperplastic change of adjacent tubules. Some foci adjacent to the capsular surface in one male at 300 mg/kg bw/d (none in controls or any females) were associated with the subcapsular vessels and had been identified as cysts at gross necropsy. In some of them, crystalline material (coded debris in the table below) occurred in association with minimal haemorrhage and acute inflammation, or with a more chronic response. Renal proximal tubular epithelial cell swelling and dissolution with minimal pycnosis (coded as degeneration in the table below) also occurred in three males at 300 mg/kg bw/day. Chronic inflammation remained in two males and one female from the treated recovery group eight weeks after the cessation of treatment.*'

There were no histopathological findings in the liver.

Summary of histopathology findings in the kidneys in 90-day dog study

Parameter	No. animals affected (mean severity) in:							
	Males treated at (mg/kg bw/day):				Females treated at (mg/kg bw/day):			
	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, 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)
- debris	0	0	1 (2.0)	2 (1.5)	0	0	2 (2.0)	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, acute	0	-	-	0	0	-	-	0
- inflammation, chronic	0	-	-	2 (2.0)	0	-	-	1 (2.0)
- debris	0	-	-	0	0	-	-	0

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

In the one-year study, doses of 0, 5, 40 and 125 mg/kg bw/d were administered. Overall body-weight gain was lower than controls at 125 mg/kg bw/d and also marginally lower in males at 40 mg/kg bw/day. ALP was increased in females treated with 125 and 40 mg/kg bw/day. Treatment-related increases in relative liver and kidney weights were recorded at 125 mg/kg bw/d. The differences from control in relative liver weight in females of the lower dose groups (15 % at 5 mg/kg bw/d and 13 % at 40 mg/kg bw/d) were not dose-related nor correlated with any histopathology changes, and hence are concluded by the dossier submitter to be not related to prothioconazole exposure.

Treatment-related histopathological changes were apparent in the liver and kidneys of both sexes at 125 mg/kg bw/day. The kidney findings were compiled in the study report summary table in rather general terms (reproduced below); the study authors described the changes as follows. '*The morphological changes in the kidney were characterised by minimal to mild focal to multifocal chronic inflammation of the renal cortex, often with extensions into the medulla. Minimal inflammatory cells, particularly lymphocytes, were also present. Adjacent tubules frequently showed compensatory hyperplastic changes. Inflammation occurred in isolated males at 40 and 125 mg/kg bw/d and in all females at 125 mg/kg bw/d. Crystalline material occurred in some foci in females at 40 and 125 mg/kg bw/d, and in one male at 125 mg/kg bw/d. An increased incidence of pigmentation was observed in kidneys of males at 40 and 125 mg/kg bw/d for which a relationship to treatment cannot be completely excluded.*' The dossier submitter notes that pigmentation was observed in one male at 40 mg/kg bw/d (severity 3.0 = moderate) and two males at 125 mg/kg bw/d, but with a lower severity grade (1.5 = minimal / slight). Renal proximal tubular epithelial degeneration was not recorded in any animal.

In the liver, pigmentation (which stained for iron and bile) was recorded in all females of the high-dose group and in one high-dose male, most prominently in the Kupffer cells. Pigmentation was not detected in the other groups. There were no dose-related findings in any organ or tissue at 5 mg/kg bw/d.

Summary of histopathological findings in the kidneys in one-year dog study

Organ / finding	No. animals affected (mean severity) in:							
	Males (mg/kg bw/day)				Females (mg/kg bw/day)			
	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
- 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

10.12.2 Dermal

A sub-acute (28-day) dermal repeated-dose toxicity study in rats is available. Doses of 100, 300 and 1000 mg/kg bw/day prothioconazole were applied to Wistar rats for 6 hours a day for 4 weeks. There were isolated incidences of erythema in 1/10 females at 100 mg/kg bw/day and 2/10 females at 1000 mg/kg bw/day, but not at 300 mg/kg bw/day. In the absence of a consistent effect these findings were attributed to mechanical

irritation at the application site as a result of the dosing procedure. There was no effect on skin thickness. Haematology and clinical chemistry values were similar between controls and treated animals. There were no effects on organ weights and no treatment-related macroscopic or microscopic findings. Therefore, it can be concluded that dermal administration of prothioconazole did not induce any systemic toxicity or adverse local effects.

10.12.3 Other studies relevant to STOT-RE

The long-term repeated-dose toxicity of prothioconazole has been investigated in rats and mice by the oral (gavage) route (see section 10.9).

Wistar rats were dosed with 0, 5, 50 and 750 mg/kg bw/day prothioconazole for one year in a chronic toxicity study and for two years in a carcinogenicity study (in this study, the high dose was reduced to 500 mg/kg bw/d in males and 625 mg/kg bw/d in females because of excessive toxicity). Adverse effects were mainly noted at the high-dose level of 750 mg/kg bw/d, which far exceeds the adjusted guidance cut-off value for classification. These effects comprised an increase in deaths, kidney toxicity (increased water consumption, increased urinary excretion, urinalysis findings and kidney histopathology), emaciation (two-year study), liver toxicity (weight increases, histopathology, clinical-chemistry changes), and an exacerbation of an age-related ocular lesion. No effects were recorded at the mid-dose level of 50 mg/kg bw/d in the one-year study, whilst the only adverse effects at this dose in the two-year study were a slight increase in the severity of chronic progressive nephropathy in males, increased urinary output and an increase in platelet counts. There were no effects at 5 mg/kg bw/d in either study.

CD-1 mice were dosed with 0, 10, 70 and 500 mg/kg bw/day for 18 months. Clinical signs were observed only in the high-dose group (piloerection, pallor and poor general condition) and near to the end of the study. Lower body-weight gains were recorded at 70 and 500 mg/kg bw/day after 4-6 weeks of treatment. As in rats, the target organs were the liver and kidneys. At 500 mg/kg bw/d, liver weights were statistically significantly increased and were associated with histopathological indications of hepatic enzyme induction and toxicity. Also at this dose, kidney weights were decreased in males and histopathology revealed adverse findings in both sexes. At the mid-dose level of 70 mg/kg bw/d, the liver weights of males were increased and hepatocellular hypertrophy with cytoplasmic change was reported, whilst an increased incidence of renal tubular degeneration / regeneration was evident in males. No adverse effects were observed at 10 mg/kg bw/d.

10.12.4 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The repeated-dose toxicity of prothioconazole by the oral route has been investigated in 28-day, 90-day and chronic studies in rats, 90-day and 18-month studies in mice and 90-day and one-year studies in dogs. Repeated-dose toxicity via the dermal route was investigated in a 28-day study in rats, in which no adverse effects were reported up to the limit dose of 1000 mg/kg bw/day.

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

Liver

The predominant effects on the liver were indicative of adaptive responses consequent to the extensive hepatic metabolism of prothioconazole: increases in relative liver weights of around 10 % or less, liver-enzyme induction and hepatocellular hypertrophy with cytoplasmic changes. This was particularly the case in rats, in which doses up to 146 / 151 mg/kg bw/d for 28 days, 500 mg/kg bw/d for 90 days and 50 mg/kg bw/d for one and two years did not result in liver toxicity. In an additional 28-day study in rats, in which a single gavage dose of 1000 mg/kg bw/d was administered, liver changes comprised only weight increases of 10-20 % in females and minimal-to-slight cytoplasmic change in centrilobular hepatocytes (consistent with hepatic enzyme induction).

In dogs, liver weights were increased by 11 % in females (no change in males) in the 90-day study at 100 mg/kg bw/day and at 125 mg/kg bw/day in the one-year study. Histopathology findings (pigmentation) were only observed in dogs in the latter study and in the high-dose group (125 mg/kg bw/day).

More severe liver effects were observed in mice. In the 90-day study, a dose-related increase in liver weights was observed from 25 mg/kg bw/d in males and from 100 mg/kg bw/day in females (up to a 56 % change in relative weight at 400 mg/kg bw/day). The increase in relative liver weight in males at 25 mg/kg bw/d was relatively small (17 %) and not associated with histopathology changes and is therefore regarded by the dossier submitter as adaptive rather than adverse. Besides hepatocellular hypertrophy and cytoplasmic changes, centrilobular (males) and periportal fatty change (females) were noted from 100 mg/kg bw/day; it is noted, however, that the incidence of centrilobular fatty change in males was also high in the control group (8/10 animals affected). Focal necrosis and overt liver lobulation were recorded in some animals of the high-dose group.

Despite the liver toxicity observed in the 90-day mouse study, prolonged administration of prothioconazole for 18 months at doses up to 500 mg/kg bw/d did not exacerbate the hepatotoxicity. At 500 mg/kg bw/d, liver weights were adversely affected (relative weights increased by 39 %) and pathology revealed lobulation (males) and indications of liver-enzyme induction, but there was no necrosis or fatty change. At the mid-dose level of 70 mg/kg bw/d, an increase in liver weight in males was associated with histopathology findings that indicated liver-enzyme induction. No liver effects occurred at the low dose of 10 mg/kg bw/d.

Kidney

Effects on the kidneys were identified in rats, mice and dogs and comprised changes in urinary output, some biochemical alterations and pathology findings.

In the short-term repeated-dose toxicity studies, histopathological changes consisted of increased incidence and severity of basophilic tubules and tubular dilatation in rats at 952/1033 mg/kg bw/day in the 28-day study and 500 mg/kg bw/day in the 90-day study. Following prolonged administration for one or two years, kidney toxicity was severe (resulting in deaths) at the high-dose of 750 mg/kg bw/d, but with no effects at the next dose of 50 mg/kg bw/d for one year; extension of the dosing period to two years resulted in some minor kidney toxicity at this dose, some of which was likely to be male-rat specific. When mice were dosed for 18 months, histopathology changes in the kidneys were evident at 70 and 500 mg/kg bw/d, but exposure for 90 days at doses up to 400 mg/kg bw/d did not result in evidence of kidney toxicity.

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; in the high-dose (300 mg/kg bw/d) recovery group, this was only partially reversed, but a mid-dose recovery group was not included. Following administration for one year of doses up to 125 mg/kg bw/d, renal chronic inflammation and pigmentation were noted in males at 40 mg/kg bw/d, although there wasn't a dose-related increase in the incidence of chronic inflammation (and the incidence in one animal in each of these groups was the same as that in the control females); the severity score of the pigmentation at 125 mg/kg bw/d was lower than that at the mid-dose level. Therefore, it's somewhat uncertain if these were treatment-related effects. It was clear, however, that a longer duration of exposure did not exacerbate or even completely replicate the renal toxicity observed in the 90-day study. For example, renal proximal tubular epithelial degeneration was reported in 3/4 males at doses of 300 mg/kg bw/d for 90-days, but not in any animals at doses up to 125 mg/kg bw/d for one year.

10.12.5 Comparison with the CLP criteria

Classification for STOT-RE is warranted when repeated exposure to a substance results in 'significant' or 'severe' toxicity, generally at doses that are around or below the reference values assigned in the guidance on the application of the CLP criteria. For a 90-day oral study in the rat, the guidance cut-off value for category 2 is ≤ 100 mg/kg bw/d; this value is adjusted to ≤ 300 mg/kg bw/d for a 28-day study and ≤ 25 mg/kg bw/d for a one-year study. For category 1, the guidance cut-off value for an oral 90-day study in rats is ≤ 10 mg/kg bw/d. In the context of classification, 'significant' is taken to mean morphological changes that are toxicologically significant, or effects that clearly indicate functional disturbance. 'Severe' refers to more profound effects of an adverse nature or effects which significantly impact on health.

No effects were observed when prothioconazole was administered dermally to rats for 28 days at doses up to 1000 mg/kg bw/d; therefore classification for STOT-RE via the dermal route is not warranted. In the available repeated-dose oral studies in rats, mice and dogs, effects on the liver and kidneys were observed around the CLP guideline values for classification for STOT-RE. These effects are summarised below.

Comparison of the effects of repeated-dose toxicity following oral administration of prothioconazole with the CLP guideline doses

Study	CLP guidance value for classification	Liver effects below guidance value	Kidney effects below guidance value
28 day rat study	Cat 1 = 30 Cat 2 = 300	Category 1 18.6/18.8 mg/kg bw/day – no effects Category 2 146/151 mg/kg bw/day – no adverse effects	Category 1 Lowest dose 18.6/18.8 mg/kg bw/day– no effects Category 2 146/151 mg/kg bw/day – no effects
90 day rat study	Cat 1 = 10 Cat 2 = 100	Category 1 Lowest dose = 20 mg/kg bw/day – no effects Category 2 100 mg/kg bw/day – no effects	Category 1 Lowest dose = 20 mg/kg bw/day – no effects Category 2 100 mg/kg bw/day – no effects
1-year rat study	Cat 1 = 2.5 Cat 2 = 25	Category 1 Lowest dose = 5 mg/kg bw/day – no effects Category 2 50 mg/kg bw/day – no effects	Category 1 Lowest dose = 5 mg/kg bw/day – no effects Category 2 50 mg/kg bw/day – no effects
2-year rat study	Cat 1 = 1.25 Cat 2 = 12.5	Category 1 Lowest dose = 5 mg/kg bw/day – no effects Category 2 50 mg/kg bw/day – no liver effects	Category 1 Lowest dose = 5 mg/kg bw/day – no effects Category 2 5 mg/kg bw/day – no effects; next dose 50 mg/kg bw/d (nephropathy and increased urinary output)
90-day mouse study	Cat 1 = 10 Cat 2 = 100	Category 1 25 mg/kg bw/day – 17 % increase in relative liver weight (males) Category 2 100 mg/kg bw/day – 21 % / 15 % ↑ in relative liver weight (males and females, respectively), centrilobular fatty change (10/10 males, but also in 8/10 controls)	Category 1 25 mg/kg bw/day – no renal effects Category 2 100 mg kg bw/day – no renal effects
18-month mouse study	Cat 1 = 1.7 Cat 2 = 17	Category 1 10 mg/kg bw/day – no effects Category 2	Category 1 10 mg/kg bw/day – no effects Category 2

Study	CLP guidance value for classification	Liver effects below guidance value	Kidney effects below guidance value
		70 mg/kg bw/day – 16 % increase in relative liver weight (males); hypertrophy and cytoplasmic changes	10 mg/kg bw/day – no effects; next dose 70 mg/kg bw/d (renal tubular degeneration / regeneration in males)
90-day dog study	Cat 1 = 10 Cat 2 = 100	Category 1 Lowest dose = 25 mg/kg bw/day – no effects Category 2 100 mg/kg bw/day – no adverse effects	Category 1 Lowest dose = 25 mg/kg bw/day – no effects Category 2 100 mg/kg bw/day –chronic inflammatory changes (3/4 males), debris (1/4 males)
1-year dog study	Cat 1 = 2.5 Cat 2 = 25	Category 1 Lowest dose = 5 mg/kg bw/day – no effects Category 2 40 mg/kg bw/day – no adverse effects	Category 1 Lowest dose = 5 mg/kg bw/day – no effects Category 2 5 mg/kg bw/day – no effects; next dose 40 mg/kg bw/d – chronic inflammation & pigmentation, although without clear dose-response relationships

No adverse effects in either the liver or kidneys were noted in any species at doses equal to or below the guidance cut-off values for STOT-RE category 1.

In terms of liver effects, the mouse was the most sensitive species of those investigated. In the 90-day mouse study, liver effects at the guidance cut-off value of 100 mg/kg bw/d comprised increases in organ weight and an increase in centrilobular fatty change in males, although the incidence of the latter was also very high in the control group. Furthermore, when administration was continued for 18 months, the liver toxicity was not exacerbated; there were no effects at 10 mg/kg bw/d, and at the mid-dose level of 70 mg/kg bw/d (which was far higher than the adjusted guidance value for category 2 of 17 mg/kg bw/d), the only observed liver effects (weight increase and hepatocellular hypertrophy and cytoplasmic change) were indicative of adaptive changes. There were no adverse liver effects in rats or dogs at doses relevant for classification. Therefore, classification for STOT-RE based on liver toxicity is not proposed.

Kidney effects were reported in the 90-day dog study at the guidance cut-off value for category 2 (100 mg/kg bw/d), comprising chronic inflammatory changes, but not in the one-year study at doses relevant for classification. Renal changes were only observed in rats and mice at doses in excess of the guidance cut-off values and so do not support classification. The renal findings in dogs are compared with the CLP criteria below to ascertain if they were significant or severe enough to warrant classification for STOT-RE.

(a) *Morbidity or death resulting from repeated or long term exposure.*

There were no deaths.

(b) *Significant functional changes in the central or peripheral nervous system or other organ systems.*

This was not a feature of prothioconazole exposure.

(c) *Any consistent or adverse change in clinical biochemistry, haematology or urinalysis parameters.*

There were no consistent or adverse changes in these parameters at doses relevant for classification.

(d) *Significant organ damage noted at necropsy.*

Histopathological renal findings at 100 mg/kg bw/day in a 90-day dog study, mainly in males, comprised chronic inflammatory changes of slight severity that were characterised by multi-focal chronic interstitial fibrosis in the cortex and medulla, inflammation (minimal) and debris (crystalline material) in one male (slight). There was no renal proximal tubular epithelial cell degeneration at this dose. At the next dose (300 mg/kg bw/d; above the guidance cut-off value for classification) in the same study, the inflammatory effects progressed slightly in severity and were also 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.

In the second available dog study, prothioconazole was administered for one year. This prolonged exposure did not result in a noticeable exacerbation of the kidney toxicity: chronic inflammatory changes were recorded in one male of each of the mid- (40 mg/kg bw/d) and high- (125 mg/kg bw/d) dose groups. The only other finding at 40 mg/kg bw/d was pigmentation in one male, although the severity had a higher score than that in the high-dose group, making the toxicological relevance of this finding unclear. The mid-dose level of 40 mg/kg bw/d was higher than the adjusted guidance cut-off value of 25 mg/kg bw/d. No effects were recorded at 5 mg/kg bw/d.

(e) *Multi-focal or diffuse necrosis, fibrosis or granular formation in organs with regenerative capacity.*

No findings at doses relevant for classification.

(f) *Morphological changes that are reversible but provide clear evidence of marked organ dysfunction.*

No relevant findings.

(g) *Evidence of cell death in organs incapable of regeneration.*

There was no evidence of appreciable cell death at doses relevant for classification.

The kidney was a clear target organ for prothioconazole following repeated oral exposure, resulting in significant toxicity in rats, mice and dogs and, at high doses for prolonged durations (≥ one year), severe toxicity in rats. However, in the majority of studies, the renal toxicity was only evident at doses that exceeded the guidance cut-off values for category 2. In only one study, a 90-day gavage study in dogs, were effects (graded as minimal to slight) observed at a dose that was relevant for classification. Extension of the dosing period to one year did not increase the incidence or severity of renal findings: adverse effects did not occur at a dose relevant for classification, whilst the absence of clear dose-response relationships in the findings at the next dose (higher than the adjusted guidance cut-off value) made their toxicological relevance uncertain. On balance, therefore, the dossier submitter proposes not to classify prothioconazole for STOT-RE.

10.12.6 Conclusion on classification and labelling for STOT RE

Not classified – Conclusive but not sufficient for classification

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 ♀: 0, 18.8, 151, 1033 mg/kg bw/d	<u>952/1033 (♂/♀) mg/kg bw/d:</u> ▪ ↓ 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 (♂,♀) <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	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.
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	No adverse effects observed at any dose	Cat 1 = 60 Cat 2 = 600	No adverse effects at any dose. Data not sufficient for classification.

0, 100, 300 and 1000 mg/kg bw/d			
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.
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	<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)	Cat 1 = 1.25 Cat 2 = 12.5	No treatment-related findings at dose levels relevant for classification.

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)	▪ ↑ 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> No findings		Data not sufficient for classification.
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%**/-)	Cat 1 = 1.7 Cat 2 = 17	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.

	▪ 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		
Dog, 90-day oral gavage, OECD TG 409, GLP 0, 25, 100, 300 mg/kg bw/day, 5 days/week. 8 week recovery period (control and high dose groups).	<u>300 mg/kg bw/day</u> ▪ ↑ rel. liver wt. (♂: +20%, ♀: +19%*) ▪ ↑ hepatic microsomal enzymes (2 fold, ♀) ▪ ↑ ALT (♂: +25%, ♀: +113%*) ▪ ↑ ALP (♂: +72%, ♀: +41%) ▪ ↓ 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	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.
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%*)	Cat 1 = 2.5 Cat 2 = 25	No treatment-related findings at dose levels relevant for classification. Data not sufficient for classification.

	▪ 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		

* Significantly different from control, p ≤ 0.05, ** Significantly different from control, p ≤ 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**.

10.13 Aspiration hazard

Table 37: Summary table of evidence for aspiration hazard

No data are available.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data are available				

No classification for aspiration hazard is proposed.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 38: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
11.1.3 Aquatic hydrolysis			
Test substance: prothioconazole Guidelines EEC method C7; SETAC; EPA 161-1. GLP compliant.	Prothioconazole: pH 9, 50°C : DT_{50} > 1 year pH 7, 50°C : DT_{50} > 1 year pH 4, 50°C : DT_{50} = 120 days; JAU 6476-desthio identified, max 5.3% (at 168 days)	Reliable (no significant deviations from the guideline)	Prothioconazole: Riegner, K., 1998. Report no. MR-623/98 Doc no. M-005117-01-1
Test substance: JAU 6476-desthio Guidelines EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate, Section 161-1 (1982) GLP compliant.	JAU 6476-desthio: Hydrolytically stable at pH 5, 7, and 9 (< 6% degradation).	Reliable (no significant deviations from the guideline)	JAU 6476-desthio: Hellpointner, E., 1993 Report no. PF3882 Doc no. M-008584-01-3
11.1.4.3 Water / sediment			
Test substance: Prothioconazole Guidelines Original study: BBA IV, 5-1, 1990; SETAC 1995 GLP compliant New kinetic assessment: FOCUS, 2014[1]	Prothioconazole: Degradation DT_{50} whole system (adjusted to 12°C): Hönniger Weiher (HW): 1.1 days Anglerweiher (AW): 2.3 days JAU 6476-desthio: Max: water 32.3% AR sediment 26.9% AR whole system 54.5% AR Degradation DT_{50} whole system (adjusted to 12°C): Hönniger Weiher: 165 days Anglerweiher: 77.0 days Total mineralisation: 14.7% AR (HW) and 29.0% AR (AW)	Reliable (no significant deviations from the guideline)	Brumhard, B and Oi, M., 2001 (main study) Report no. PF3852 Doc no. M-034440-02-1 Chapple, A.C.;Hoerold, C., 2015 (kinetic assessment) Report no. EnSa-14-1115 Doc no. M-534364-01-1
11.1.4.3 Aerobic mineralisation			
Test substance: Prothioconazole Guidelines Original study: OECD 309 GLP compliant.	Prothioconazole: Degradation DT_{50} values at different concentrations (adjusted to 12°C): 10 µg/L: 160 days 100 µg/L: > 1000 days JAU 6476-desthio: Maximum formation: 41.9% AR	Reliable (no significant deviations from the guideline)	Heinemann, O.; Junge, T.; 2014 (main study) Report no. PF3852 Doc no. M-496435-01-1 Chapple, A.; Hoerold, C.; 2015 (kinetic assessment)

Method	Results	Remarks	Reference
New kinetic assessment: FOCUS, 2014[1]	Total mineralisation: ≤ 0.5% AR		Report no. EnSa-15-0389 Doc no. M-531380-01-1
11.1.4.3 Anaerobic water / sediment			
Test substance: Prothioconazole Guidelines EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate, Section 162-3 (1982) GLP compliant.	Prothioconazole: Degradation DT_{50} whole system (adjusted to 12°C): 92.0 days JAU 6476-S-methyl: Max: water 8.6% AR sediment 77.1% AR whole system 77.1% AR	Reliable (no significant deviations from the guideline)	Scholz, K.; 2001. Report no. MR-275/01 Doc no.: M-137101-01-1
11.1.4.4 Photochemical degradation			
Test substance: Prothioconazole Guidelines Original study: EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate, Section 161-1 (1982). New kinetic assessment: FOCUS, 2014[1]	Prothioconazole: Experimental DT_{50}: 2.1 days Environmental DT_{50}: 11.5 days JAU 6476-desthio: Experimental DT_{50}: 54.8 days Environmental DT_{50}: 307 days Maximum formation: 54.9% AR Total mineralisation: 3.0% AR	Reliable (no significant deviations from the guideline)	Gilges, M.; Bomatsch, W., 2001. Report no. MR-213/01 Doc no. M-064326-01-1 Chapple, A.; Hoerold, C.; 2015 (kinetic assessment) Report no. EnSa-15-0265 Doc no. M-532628-01-1
Test substance: Prothioconazole Guideline: ECETOC method (1981, 1984), Test Guideline 'Phototransformation of chemicals in water, Part A (Berlin, 1992)	Prothioconazole: Quantum yield: 0.0638 (pH 4.0) 0.0047 (pH 9.0) Environmental direct photolysis half-lives: pH 4: 50 - > 200 days pH 9: 7 – 20 days JAU 6476-desthio: Quantum yield: 0.00449 (in high purity water)	Reliable (no significant deviations from the guideline)	Prothioconazole: Hellpointner, E. (2001) Report no. MR-101/01 Doc no. M-051279-01-1 Hellpointner, E. (2001) Report no. PF3852 Doc no. M-008540-01-1

11.1.1 Ready biodegradability

A study on the "ready biodegradability" of prothioconazole was not performed.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

The hydrolysis of prothioconazole was investigated in the dark at 50°C in aqueous buffered solutions at either pH 4, pH 7, or pH 9. 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 (5.3%) and other degradants were 4.2%. There was less than10% degradation of active substance over the course of the 7 day study at all pH values tested. Therefore, prothioconazole is considered stable to hydrolysis and hydrolytic breakdown is not expected to contribute to its degradation in the environment.

JAU 6476-desthio was hydrolytically stable with a degradation DT_{50} value greater than 1 year at all pH values tested. Hydrolysis is unlikely to play a significant role in the environmental fate of JAU 6476-desthio.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Aerobic water / sediment

A water / sediment study was conducted using two natural systems called Hönniger Weiher (HW) and Anglerweiher (AW) at 20°C for a period of 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 unextracted 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 carbon dioxide 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 maximums 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 geomean 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). A summary of values for each system summarised below, all adjusted to 12°C.

System	DT_{50} values (adjusted to 12°C)			
	Prothioconazole		JAU 6476-desthio	
	Water dissipation	Whole system degradation	Water dissipation	Whole system degradation
Hönniger Weiher	0.44	1.1	5.7	165
Anglerweiher	1.2	2.3	58.2	77.0
Geomean	0.73	1.6	18.2	113

Aerobic mineralisation

The degradation of prothioconazole was studied in surface water under aerobic conditions for 60 days at 19.3°C using nomnal test concentrations of either 10 µg/L or 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 > 1000 days (high concentration).

Water / sediment system (anaerobic)

The fate of prothioconazole in an anaerobic water/sediment system at 20°C was investigated for 360 days after application of prothioconazole. During the study radioactivity in the water layer decreased to 1.4% AR, unextracted residue increased to 27.5% AR, and CO_2 was always less than 0.1% AR. Prothioconazole in the water layer was less than 10% AR within 30 days and amounts in the sediment peaked at 52.2% AR after 91 days and then declined to < LOD. The metabolite JAU 6476-S-methyl reached a maximum of 8.6% AR in the water after 30 days but was < LOD by 91 days. It increased in the sediment to to 77.1% AR after 240 days and was still 76.1% AR at the end of the study. The water phase dissipation DT_{50} value for prothioconazole was 4.9 days and the whole system degradation DT_{50} value was 93.5 days (both values adjusted to 12°C).

11.1.4.4 Photochemical degradation

Aqueous photolysis

Aqueous photolytic degradation was investigated in a pH 7 buffer solution at 25°C using xenon light with a 290 nm filter and continuous exposure for 18 days, equivalent to 100.7 days in Athens (Greece). 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) were detected as major degradants. There was some evidence of 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).

Quantum yield

Mean quantum yields (Φ)were 0.0638 (pH 4) and 0.0047 (pH 9) for prothioconazole and 0.00449 for JAU 6476-desthio.

11.1.5 Overall summary on environmental degradation

Prothioconazole

Prothioconazole did not hydrolyse at pH 7 or 9 and at pH 4 there was only a small amount of hydrolysis. It was completely degraded by photolysis, the main metabolite formed being JAU 6476-desthio. The experimental degradation DT_{50} was 2.1 days. However, the environmental relevance of photolysis in relation to meeting CLP criteria for 'rapid degradability' is uncertain. It was partially degraded in the aerobic mineralisation study, again forming JAU 6476-desthio as the main metabolite . The experimental degradation DT_{50} was 160 days (low concentration, adjusted to 12°C). Prothioconazole dissipated rapidly from the water phase of the water / sediment system (geomean dissipation DT_{50} = 0.73 days, adjusted to 12°C). Loss from the whole system was also rapid, the whole system geomean degradation DT_{50} being 1.6 days (adjusted to 12°C). JAU 6476-desthio was a major metabolite formed and there were four other degradants at greater than 5% AR.

JAU 6476-desthio

JAU 6476-desthio was stable to hydrolysis at pH 5, 7, and 9 (degradation DT_{50} value > 1 year). Degradation of JAU 6476-desthio did occur in the photolysis study where it was first formed from prothioconazole. The degradation DT_{50} value was 54.8 days. It was formed from prothioconazole in the aerobic mineralisation study but there was no clear evidence for degradation. JAU 6476-desthio was rapidly formed in the water / sediment study (maximum 23.4% AR in water, 32.3% AR in sediment) and was lost from the water phase with a dissipation DT_{50} value of 18.2 days (adjusted to 12°C). The whole system degradation DT_{50} value was 113 days (adjusted to 12°C).

Summary

No ready biodegradability test was carried out and prothioconazole fails the test for rapid degradability based on hydrolysis and aerobic mineralisation. The whole system degradation half-life in the water / sediment system was 1.6 days and therefore fulfils the criterion that there is greater than 70% degradation in 28 days. However, the principal degradation product JAU 6476-desthio is classified as hazardous to the aquatic environment. In addition the degradant 1,2,4-triazole was formed in water but there was no clear evidence of degradation. Therefore, it is concluded that although prothioconazole degrades quickly in the whole system of the water / sediment study, it does not meet the CLP criteria for 'rapid degradability'.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for classification of prothioconazole

11.2.1 Summary of data/information on environmental transformation

Not relevant for classification of prothioconazole

11.3 Environmental fate and other relevant information

Table 39: Summary of relevant information on environmental fate and other relevant information

Method	Results	Remarks	Reference
Mobility in soil			
Test substance: Prothioconazole Guideline:	Prothioconazole: estimated K_d: 15.2 mL/g estimated K_{oc}: 1765 mL/g	Reliable (Koc estimated from this study;	Babczinski, P., 2001 Report no. MR-364/00 Doc no. M-055836-02-1

Method	Results	Remarks	Reference
EPA 1631-1 GLP compliant		non standard method)	
Test substance: JAU 6476-desthio Guideline: OECD 106 GLP compliant	JAU 6476-desthio Kd: 4.13 to 13.38 mL/g; geomean: 8.85 mL/g Koc: 523 to 625 mL/g; geomean: 574 mL/g	Reliable (no significant deviations from the guideline)	Fent, G. Report no. FM768 Doc no. M-008501-01-1
Photochemical oxidative degradation in air:			
Test substance: Prothioconazole Guideline: BBA, Part IV, 6-1 non-GLP	Prothioconazole: Half-life in air: 1.1 hours Maximum chemical lifetime in air: 3 hours	Reliable (no significant deviations from the guideline)	Hellpointner, E., 1999. Report no. MR-093/99 Doc no. M-1450958-2
Test substance: JAU 6476-desthio Guideline: BBA, Part IV, 6-1 non-GLP	JAU 6476-desthio: Half-life in air: 14.2 hours Maximum chemical lifetime in air: 23 hours	Reliable (no significant deviations from the guideline)	Hellpointner, E., 1999. Report no. MR-323/00 Doc no. M-1451066-3

Mobility in soil: aged residue column leaching

K_d and K_{oc} values of prothioconazole could not be determined in batch equilibrium studies due to the instability of the compound in these systems. Therefore, a parent aged residue column leaching study was perfomed. The aged leaching study offered the possibility to estimate a K_d value from the leaching behaviour of prothioconazole in a soil column. The calculated K_d value of 15.2 mL/g resulted in a calculated K_{oc} value of 1765 mL/g of prothioconazole in loamy sandy soil.

The adsorption/desorption of JAU 6476-desthio was determined by a batch adsorption method for four soils. The calculated adsorption K_{oc} was in the range 523 – 625 mL/g, geomean 574 mL/g and the mean calculated Freundlich exponent (1/n) was 0.81.

Photochemical oxidative degradation in air

The chemical lifetime of prothioconazole in the air was assessed using the Atkinson calculation and assumed a 12-hour day and $1.5x10^6$ OH radicals/cm^3. The assessment indicated a chemical half-life in air of 1.1 hours and a maximum chemical lifetime in air for prothioconazole of about 3 hours. A similar assessment was carried out for JAU 6476-desthio, and the chemical half-life in air of 14.2 hours with a maximum chemical lifetime in air of 23 hours. It can be concluded that neither prothioconazole nor JAU 6476-desthio would be expected to be transported in the gaseous phase over large distances or to accumulate in the air.

11.4 Bioaccumulation

Table 40: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Prothioconazole			
n-Octanol/water partition coefficient,	Partition coefficient of PAI in different buffered media at 25 °C: **Media** **Pow** **log Pow**	The results show that the partition coefficient of prothioconazole is highly pH dependent.	Ziemker & Strunk, 2014

Method	Results	Remarks	Reference
OECD 117, EC A. 8	buffer pH 4 2512 3.4 buffer pH 7 100 2.0 buffer pH 9 1.6 0.2		
Experimental aquatic BCF OECD Guideline 305, GLP	$BCF_{(whole\ fish,\ wet\ weight)}$: 43.9 - 57.8 $BCF_{(whole\ fish,\ normalised\ to\ 6\%\ lipid\ content)}$: 18.8	28 days with constant exposure and 14 days of depuration, flow-through	Anonymous, 2001j
Prothioconazole-desthio			
n-Octanol/water partition coefficient, OECD 117, EC A. 8	Partition coefficient in unbuffered demineralized water at 22 °C: **Media** **Pow** **log Pow** Unbuffered demineralized water 1100 3.04	-	Krohn, J.; 1992
Experimental aquatic BCF OECD Guideline 305, GLP	$BCF_{(whole\ fish,\ wet\ weight)}$: 71.6 - 94.3 $BCF_{(whole\ fish,\ normalised\ to\ 6\%\ lipid\ content)}$: 45	28 days with constant exposure and 14 days of depuration, flow-through	Anonymous 2001

11.4.1 Estimated bioaccumulation

Prothioconazole has a log P_{OW} of 2.0 at pH 7 (see section 7 and Tables 7 and 40), which indicates that this has a low bioaccumulation potential. A bioaccumulation study is available that shows that prothioconazole does not bioaccumulate (see section 11.4.2).

Prothioconazole-desthio has a log P_{OW} of 3.04 (see section section 7 and Tables 7 and 40), which indicates that this main degradant also has a low bioaccumulation potential. A bioaccumulation study is available that shows that prothioconazole-desthio does not bioaccumulate (see section 11.4.2).

11.4.2 Measured partition coefficient and bioaccumulation test data

Prothioconazole

The partion coefficient 1-octanol/water of prothioconazole was determined in pH 4, pH 7 and pH 9 according to OECD Guideline 117 (Ziemker & Strunk, 2014, M-492539-01-1). Nine neutral calibration substances were injected into an HPLC-system under the same analytical conditions as the test item (column temperature 25°C). Calibration curves were created by using the measured retention times (log k'-values) and the known log P_{OW}-values of the calibration substances for linear regression. From the resulted calibration curves and their equations the log P_{OW} values of the test item were interpolated for pH 4 and pH 7 and extrapolated for pH 9. Mean log P_{ow} values were 3.4, 2.0, 0.2 at pH 4, 7 and 9, respectively. The results show that the partition coefficient of Prothioconazole is highly pH dependent with a low bioaccumulation potential.

Anonymous (2001j) investigated bioconcentration of phenyl labelled prothioconazole bluegill sunfish (*Lepomis macrochirus*) over 28 days. A flow-through test system was used to maintain mean water concentrations of 5 µg and 50 µg ^{14}C-prothioconazole/L. After the exposure of 28 days, the test fish were placed in clean water for 14 days in order to determine the depuration of ^{14}C-prothioconazole. In a second test, 30 bluegill sunfish were exposed to 50 µg ^{14}C-prothioconazole/L for 7 and 14 days to investigate biotransformation of prothioconazole. The test fish of the second experiment were sampled and divided into edible and viscera tissues after 7 days and 14 days, respectively. Prothioconazole accumulates rapidly in bluegill sunfish with a total residue bioconcentration factor of 43.9 to 57.8 for whole fish. When exposure ceases, the residues are depurated with a half-life of 0.47 - 0.80 days. After 14 days in uncontaminated water 91 % (nominal concentration of 5 µg/L) and 95 % (nominal concentration of 50 µg/L), respectively, of the

mean plateau radioactivity were depurated from whole fish. The steady-state-BCF for prothioconazole (normalised to 6% lipid content in fish) is 18.8.

Prothioconazole-desthio

The partion coefficient 1-octanol/water of prothioconazole-desthio was determined to be 1100 (mean log P_{ow} = 3.04) at 22ºC in unbuffered water. The determination was carried out by the shake-flask method according to OECD Guideline 117 (Krohn, J., 1992, M-010758-01-1).

Anonymous (2001) investigated bioconcentration of phenyl labelled prothioconazole-desthio (>99% radiochemical purity) in bluegill sunfish (*Lepomis macrochirus*) over 28 days. A flow-through test system was used to maintain mean water concentrations of 10 µg and 100 µg ^{14}C-prothioconazole-desthio/L. After the exposure of 28 days, the test fish were placed in clean water for 14 days in order to determine the depuration of ^{14}C-prothioconazole-desthio. Prothioconazole-desthio accumulates rapidly in bluegill sunfish with a total residue kinetic bioconcentration factor of about 71.6 to 94.3 X for whole fish and 36.5 - 37.5 X for edible parts. The steady-state-BCF for prothioconazole-desthio (normalised to 6% lipid content in fish) is 45. When exposure ceases, the residues are depurated with a half-life of 0.39 - 0.47 days. After 14 days in uncontaminated water 96 % (nominal concentration of 10 µg/L) and 99 % (nominal concentration of 100 µg/L), respectively, of the mean plateau radioactivity were depurated from whole fish.

11.5 Acute aquatic hazard

A summary of all the relevant and reliable information on the acute aquatic toxicity of prothioconazole and prothioconazole-desthio is presented in Table 41. Studies were conducted according to internationally agreed standard test guidelines and corresponding validity criteria were met. In the following sections, executive summaries of the available studies on prothioconazole and prothioconazole-desthio are provided that give more detailed information on acute aquatic toxicity.

Studies with prothioconazole were carried with technical material of 97.5 – 98.8 % purity. Studies with prothioconazole-desthio were carried with technical material of 93.7 – 98.8 % purity.

Table 41: Summary of reliable information on acute aquatic toxicity

Method	Species	Test material	Results		Remarks	Reference
			Endpoint	Toxicity (mg a.s./L)		
Acute toxicity to fish, OECD Guideline 203, GLP	*Oncorhynchus mykiss*	a.s.	LC_{50}	1.83 (mm)	96 h, static	Anonymous, 1999g
Acute toxicity to fish, OECD Guideline 203, GLP	*Lepomis macrochirus*	a.s.	LC_{50}	4.59 (mm)	96 h, static	Anonymous, 1999h
Acute toxicity to fish, OECD Guideline 203, GLP	*Cyprinus carpio*	a.s.	LC_{50}	6.91 (mm)	96 h, static	Anonymous, 2000d
Acute toxicity to fish, OECD Guideline 203, GLP	*Cyprinodon variegatus*	a.s.	LC_{50}	>10.3 (mm)	96 h, static-renewal	Anonymous, 2004c

Daphnia sp Acute Imobilisation, OECD Guideline 202, GLP	*Daphnia magna*	a.s.	EC_{50}	1.3 (nom^)	48 h, static	Heimbach, 1999c
Mysid Acute, Toxicity Test, OPPTS 850.1035, GLP	*Americamysis bahia*	a.s.	LC_{50}	2.4 (mm)	96 h, flow-through	Drottar et al., 2002a
Oyster Acute Toxicity Test (Shell Deposition) OPPTS 850.1025, GLP	*Crassostrea virginica*	a.s.	EC_{50}	2.9 (mm)	96 h, flow-through	Drottar et al., 2001
Freshwater Algal Growth, Inhibition, OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata*	a.s.	72 hour E_rC_{50}	2.18 (im^)	96 h, static	Dorgerloh, 2000b
Growth and reproduction of aquatic plants, USEPA Guideline 123-2 (checked against OECD 201), GLP	*Skeletonema costatum*	a.s.	E_rC_{50}	**0.03278 (mm)***	72 h, static	Kern & De Haan, 2004
Lemna sp., Aquatic plant toxicity, OPPTS Number 850.4400 (checked against OECD 221), GLP	*Lemna gibba*	a.s.	E_rC_{50}	>0.1776 (mm)	7 d, static-renewal	Kern et al., 2004b
Acute toxicity to fish, OECD Guideline 203, GLP	*Oncorhynchus mykiss*	Prothioconazole-desthio	LC_{50}	6.63 mg p.m./L (nom^)	96 h, static	Anonymous (1990)
Acute toxicity to fish, OECD Guideline 203, GLP	*Leuciscus idus melanotus*	Prothioconazole-desthio	LC_{50}	10.4 mg p.m./L (mm)	96 h, static	Anonymous (1991)
Acute toxicity to fish, OECD EPA-FIFRA 72-1 (checked against OECD 203) GLP	*Pimephales promelas*	Prothioconazole-desthio	LC_{50}	11.4 mg p.m./L (mm)	96 h, static-renewal	Anonymous (2003c)
Daphnia sp Acute Imobilisation, OECD	*Daphnia magna*	Prothioconazole-desthio	EC_{50}	>10 mg p.m./L (nom^)	48 h, static	Heimbach (1990a)

Guideline 202, GLP						
Mysid Acute, Toxicity Test, OPPTS 850.1035, GLP	*Americamysis bahia*	Prothioconazole-desthio	LC_{50}	**0.060 mg p.m./L (mm)**	96 h, flow-through	Drottar *et al* (2002b)
Mysid Acute, Toxicity Test, OPPTS 850.1035, GLP	*Americamysis bahia*	Prothioconazole-desthio	LC_{50}	>1.01 mg p.m./L (mm)	96 h, flow-through	Blankinship *et al* (2003)
Crayfish acute, toxicity test, OPPTS 850.1075, GLP	*Procambarus clarkii*	Prothioconazole-desthio	LC_{50}	>26 mg p.m./L (mm)	96 h, static-renewal	Sayers (2004)
Lemna sp., Aquatic plant toxicity, OPPTS Number 850.4400 (checked against OECD 221), GLP	*Lemna gibba*	Prothioconazole-desthio	E_rC_{50} E_rC_{10}	0.0809 (mm) 0.01568 (mm)	7 d, static-renewal	Kern et al., 2003

mm: mean measured concentration, im: initial measured concentration, nom: nominal concentration

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

* 72 hour endpoint provisional as was estimated by the RMS using 96 hour measured concentrations and the ratio between 96 hour nominal and mean measured concentration endpoints.

11.5.1 Acute (short-term) toxicity to fish

Prothioconazole

Anonymous 1999g was a 96 hour static acute toxicity test carried out on *Oncorhynchus mykiss.* The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. The LC_{50} = 1.83 mg a.s./L.

Anonymous 1999hb was a 96 hour static acute toxicity test carried out on *Lepomis macrochirus*. The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. The LC_{50} = 4.59 mg a.s./L.

Anonymous 2000d was a 96 hour static acute toxicity test carried out on *Cyprinus carpio.* The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. The LC_{50} = 6.91 mg a.s./L.

Anonymous 2004c was a 96 hour static-renewal acute toxicity test carried out on *Cyprinodon variegatus.* The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. Measurements were only made in fresh test media on days 0 and 2 and of the spent media only on day 4, i.e. not in spent media on day 2. Measurements in the fresh day 2 media and spent media on day 4 showed that minimal degradation occurred over 48 hours in this test set up and the measurements of the fresh media at day 0 showed that the concentrations were achieved within 80-120% of nominal and a level that was similar to the fresh media at day 2. Therefore, it was considered that concentrations of active substance would have been maintained within acceptable limits over the initial 48h of the study. The LC_{50} is > 10.3 mg a.s./L.

Four acute fish toxicity studies are available for prothioconazole, these were carried out on *Oncorhynchus mykiss* (Anonymous 1999g), *Lepomis macrochirus* (Anonymous 1999hb), *Cyprinus carpio* (Anonymous, 2000d) and *Cyprinodon variegatus* (Anonymous, 2004c). All the endpoints were considered reliable and all were based on mean measured concentrations. The lowest available endpoint is LC_{50} = 1.83 mg a.s./L for *Oncorhynchus mykiss*.

Prothioconazole-desthio

Anonymous, 1990 was a 96 hour static acute toxicity test carried out on *Oncorhynchus mykiss*. The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met. The endpoints were based on nominal concentrations because the measured concentrations were all maintained within ± 20% of the nominal. The LC_{50} = 6.63 mg p.m./L.

Anonymous, 1991 was a 96 hour static acute toxicity test carried out on *Leuciscus idus melanotus*. The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met. Some precipitate was seen in all except the highest nominal concentration group on day 0. However, more of the metabolite was in solution on days 1-4 in all the groups, with measured concentrations being nearly 80% or above 80% of nominal at all later measurements. The dossier submitter has calculated the mean measured concentrations for all the test groups, except for the highest concentration, which is based on the initial measured concentration. Considering that there was no mortality in the second highest concentration and 100% mortality in the highest tested concentration, it is appropriate to estimate the LC_{50} by calculating the geometric mean of these two concentrations. Therefore, the dossier submitter dossier submitter calculates that the geometric mean of 6.32 and 17.2 mg p.m./L is 10.4 mg p.m./L. The LC_{50} = 6.63 mg p.m./L.

Anonymous, 2003c was a 96 hour static-renewal acute toxicity test carried out on *Pimephales promelas*. The study was carried out according to OECD 203 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. Measurements were only made in fresh test media on days 0 and 2 and of the spent media only on day 4, i.e. not in spent media on day 2. Measurements in the fresh day 2 media and spent media on day 4 showed that minimal degradation occurred over 48 hours in this test set up and the measurements of the fresh media at day 0 showed that the concentrations were achieved within 80-120% of nominal and a level that was similar to the fresh media at day 2. Therefore, it was considered that concentrations of prothioconazole-desthio would have been maintained within acceptable limits over the initial 48h of the study. The LC_{50} is = 11.4 mg p.m./L.

Three acute fish toxicity studies are available for prothioconazole-desthio, these were carried out on *Oncorhynchus mykiss* (Anonymous, 1990), *Leuciscus idus melanotus* (Anonymous, 1991) and *Pimephales promelas* (Anonymous, 2003c). All the endpoints were considered reliable; one was based on nominal concentrations and two were based on mean measured concentrations. The lowest available endpoint is LC_{50} = 6.63 mg p.m./L for *Oncorhynchus mykiss*.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Prothioconazole

Heimbach, 1999c was a 48 hour static acute toxicity test carried out on *Daphnia magna*. The study was carried out according to OECD 202 and in compliance with GLP. All validity criteria were met. The endpoints were based on nominal concentrations. Concentrations were measured at day 0 and day 2. The day 0 measurements were all within ±20% of nominal concentrations. After 2 days, all except one tested concentration was still within ±20% of nominal concentrations. Only the lowest tested concentration was below 80% of nominal (78%), but as this was only marginally below and as no mortality occurred in this concentration it is considered that this does not affect the reliability of the endpoints. The EC_{50} = 1.3 mg a.s./L.

Drottar *et al* 2002a was a 96 hour flow through acute toxicity test carried out on *Mysidopsis bahia*. The study was carried out according to OPPTS Guideline 850.1035 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. The LC_{50} = 2.4 mg a.s./L.

Drottar *et al* 2001 was a 96 hour flow through acute toxicity test carried out on *Crassostrea virginica*. The study was largely carried out according to OPPTS Guideline 850.1025 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. Only one replicate was used for each group and not 2, as is recommended by the guideline. There is therefore uncertainty over the reliability of the endpoint. It is noted that there was a high level of variability observed in all groups, including both the control groups. Whilst this might reduce the sensitivity of the test to detect effects, it is noted that there is a dose response effect. The EC_{50} = 2.9 mg a.s./L.

Three acute aquatic invertebrate toxicity studies are available for prothioconazole, these were carried out on *Daphnia magna* (Heimbach, 1999c), *Americamysis bahia* (Drottar *et al* 2002a) and *Crassostrea virginica* (Drottar *et al* 2001). All the endpoints were considered reliable; the endpoint from Heimbach (1999c) was based on nominal concentrations, whereas the endpoints from Drottar *et al* 2002a and 2001 were based on mean measured concentrations. The lowest available endpoint is EC_{50} = 1.3 mg a.s./L for *Daphnia magna*.

Prothioconazole-desthio

Heimbach 1990a was a 48 hour static acute toxicity test carried out on *Daphnia magna.* The study was carried out according to OECD 202 and in compliance with GLP. All validity criteria were met. The endpoints were based on nominal concentrations. Concentrations were measured at day 0 in all concentrations and day 2 in only one of the concentrations. The day 0 measurements were all within ±20% of nominal concentrations, except for the highest tested concentration, which was 78% of the nominal. After 2 days, the measured concentration in the 3.2 mg p.m./L group was 100% of the nominal, i.e. the same as measured at day 0. Mortality was significantly less than 50% after 48 hours exposure in all tested concentrations. Due to precipitation occurring at the highest tested concentration and 27% mortality occurring in this group, the EC_{50} was conservatively based on the second highest concentrations tested, i.e. 10 mg p.m./L, at which 7% mortality occurred. The EC_{50} was determined to be >10 mg p.m./L. No analysis was carried out on this group after 48 hours exposure, therefore the stability of the metabolite over this period was not confirmed in this study. The EC_{50} > 10 mg p.m./L.

Drottar *et al* 2002 was a 96 hour flow through acute toxicity test carried out on *Mysidopsis bahia.* The study was carried out according to OPPTS Guideline 850.1035 and in compliance with GLP. All validity criteria were met and the endpoints were based on mean measured concentrations. The LC_{50} = 0.069 mg p.m../L.

Blankinship 2003 was a 96 hour flow through acute toxicity test carried out on *Mysidopsis bahia.* The study was carried out according to OPPTS Guideline 850.1035 and in compliance with GLP. All validity criteria were met. There was a problem with the flow-through system during the study which interrupted the flow of stock solution into all aquaria, which resulted in no test substance being delivered for a maximum of 3 hours. It is therefore considered that this is a 93 hour toxicity test. There was either very low or no mortality in all the test item groups, including no mortality at the highest concentration. Although this was not ideal, it is concluded that a further 3 hours of exposure would not result in mortality increasing to over 50% in any of the tested groups, including the highest tested concentration. Only three of the five concentrations were measured for actual test concentrations; however, as there was no mortality in the highest tested concentration, which was analytically verified, thereby leading to an unbound LC_{50} endpoint based on a concentration that was analytically measured, this does not affect the reliability of the endpoint. However, it is noted that there were no analytical measurements were made during the time when the interruption of stock solution flow to the diluter system for all treatment levels occurred. Therefore, the endpoint would be lower if it had been based on mean measured concentrations which included the lower concentrations measured during this interruption. The LC_{50} > 1.009 mg p.m../L.

Sayers 2004 was a 96 hour static-renewal acute toxicity test carried out on *Procambarus clarkii*. The study was carried out based on OPPTS Guideline 850.1075 and in compliance with GLP. No validity are available for this test species. The endpoints were based on mean measured concentrations. The dossier submitter considers the range of starting size to be large and there was no information on the age of the organisms. The size of the individuals could affect the sensitivity to the test item. Mortality was observed in all the groups, including both the control groups, which was reportedly due to cannibalisation. It is not ideal to have mortality in the control groups of 20%; however, there was no dose response so it is accepted that the mortality was not treatment related. As a general point, it would be preferable to employ a testing set up that avoids the issue of cannibalization. The oxygen saturation dropped to below 60% in several replicates at 48 hours; this adds to the uncertainty over reliability of this sudy. The actual concentrations were not measured in the 48 hour aged solutions, however, the results from the 48 hour new solutions and 96 hour aged solutions indicate that prothioconzole-desthio did not dissipate in this test system over the course of 48 hours. Furthermore, as the actual concentrations were measured at test start, the calculated mean measured concentrations are considered to be suitably accurate estimates of the exposure in the test. Overall, this study is considered to be reliable however, there is some uncertainty over the endpoint due to high variability in the starting size of test organisms and high mortality in the control group. The LC_{50} = 0.069 mg p.m../L.

Four acute aquatic invertebrate toxicity studies are available for prothioconazole-desthio, these were carried out on *Daphnia magna* (Heimbach 1990a), *Americamysis bahia* (Drottar et al 2002 and Blankinship 2003) and *Procambarus clarkii* (Sayers 2004). All the endpoints were considered reliable; however there was some uncertainty over the endpoint for *Procambarus clarkii*, which will be considered further in the below discussion. The *Daphnia magna* endpoint was based on nominal concentrations and the other three were based on mean measured concentrations.

There were two endpoints available for *Americamysis bahia;* both were from reliable studies. One endpoint was much lower that the other; however, there was no clear reason why this was. As both the studies were reliable and as there are not enough endpoints to calculate a geometric mean for use in classification, the lower endpoint will be considered for classification.

Despite the uncertainty over the endpoint for *Procambarus clarkii* this does not affect the classification because this is clearly not the most actuely sensitive aquatic invertebrate species.

The lowest available endpoint for acute toxicity to aquatic invertebrates is EC_{50} for *Americamysis bahia*, which is 0.060 mg p.m./L.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Algae

Prothioconazole

Dorgerloh, 2000b was a 96 hour static algal growth toxicity test carried out on *Pseudokirchneriella subcapitata*. The study was carried out in accordance with OECD 201 and in compliance with GLP. All validity criteria were met. The measured concentrations were all within 80-120% of nominal on day 0, but were <80% of nominal on day 4. The endpoints were based on initial measured concentrations. At test end, the highest four tested concentrations were >80% of initial measured and the lowest two tested concentrations were 70 and 74 % of initial measured concentrations. There were no effects on algal biomass or growth rate at the lowest tested concentrations. Therefore, basing the endpoints on initial measured concentrations will not impact on the reliability of the endpoints. The EC_{50} = 2.18 mg a.s./L.

Kern *et al*, 2004a was a 96 hour static algal growth toxicity test carried out on *Anabaena flos-aquae*. The study was carried out in accordance with OECD 201 and in compliance with GLP. Not all validity criteria were met. Furthermore, there appeared to be a negative effect of the solvent control, against which the test

item groups were compared. There was no dose response in the test item groups, but the test item groups were not consistently comparable to the solvent control either. Therefore, it is considered likely that the solvent control interfered with the growth of the algae. It is considered that the results of this study are not reliable. Concentrations were maintained in the three highest nominal concentrations, but in the three lowest nominal concentrations the actual concentrations dropped to below 80 % of the nominal after 96 hours. Despite this drop in concentrations the endpoints were based on initial measured concentrations. Therefore, the endpoints from this study should not be used.

Kern and Lam, 2004 was a 96 hour static algal growth toxicity test carried out on *Navicula pelliculosa*. The study was carried out in accordance with OECD 201 and in compliance with GLP. Not all the validity criteria were met; the section-by-section growth rate coefficients of variation for the negative and solvent controls were more than the criterion threshold of 10%. However, the other two validity criteria were met. Looking at the biological results data, it can be seen that that there was a lag during the first 24 hours in three of the four replicates in both control groups and in all replicates of all the test item groups. OECD 201 states the following,

'…a lag phase can be minimised and practically eliminated in control cultures by proper propagation of the pre-culture.'

This would indicate that 'proper propagation' had not occurred, therefore the endpoints from this study should not be used.

Kern and DeHaan, 2004 was a 96 hour static algal growth toxicity test carried out on *Skeletonema costatum*. The study was carried out in accordance with OECD 201 and in compliance with GLP. All the validity criteria were met except the control and solvent control mean section-by-section growth rate coefficients of variation were not <35% at 96 hours. Therefore, the 96 hour endpoints are not considered to be reliable. The 72 hour validity criteria were all met, therefore the endpoints should be based on the effects at 72 hours. The dossier submitter has estimated the 72 hour endpoints. For the EC_{50}, the ratio between the 96 hour nominal concentration E_rC_{50} and the mean measured concentration E_rC_{50} was used. As the EC_{10} and EC_{20} values were only calculated for 96 hours based on mean measured concentration, the 72 hour EC_{10} and EC_{20} values have been calculated using the the ratio between the 72 hour and 96 hour EC_{50} endpoints based on mean measured concentrations (see Tables 42 and 43). The dossier submitter acknowledges that this is not a completely accurate method for calculating the endpoints, however the resulting endpoints are considered to be reasonably conservative.

Table 42: Estimation of 72 hour mean measured concentration E_rC_{50}

96 hours			72 hours		
Nominal E_rC_{50} (µg a.s./L)	Mean measured E_rC_{50} (µg a.s./L)	Ratio between nominal and mean measured endpoints	Nominal E_rC_{50} (µg a.s./L)	Calculation	Estimated mean measured E_rC_{50} (µg a.s./L)
49.9	35.87	0.719	45.6	45.6 x 0.719	32.78

Table 43: Estimation of 72 hour mean measured concentration E_rC_{10} and E_rC_{20} values

Endpoint	96 hours		72 hours	
	Mean measured endpoint (µg a.s./L)	Ratio between 72 hour and 96 hour EC_{50} endpoint	Calculation	Estimated mean measured endpoint (µg a.s./L)
EC_{10}	15.62	0.9139	15.62 x 0.9139	14.27
EC_{20}	20.84		20.84 x 0.9139	19.04

One of the replicates in the control group experienced abnormally low growth and was thus excluded from the calculations, therefore only 2 control replicates were valid. All three solvent control replicates demonstrated very strong growth and therefore the control data were pooled, therefore, the limited number of valid negative control group replicates is not considered to impact on the validity of the endpoints. The 72 hour E_rC_{50} = 0.03587 mg a.s./L and the E_rC_{10} = 0.01427 mg a.s./L.

Four algal growth toxicity studies are available for prothioconazole, these were carried out on *Pseudokirchneriella subcapitata* (Dorgerloh, 2000b), *Anabaena flos-aquae* (Kern *et al*, 2004a), *Navicula pelliculosa* (Kern and Lam, 2004) and *Skeletonema costatum* (Kern and DeHaan, 2004).

The endpoints resulting from Kern and DeHaan (2004) are the lowest available algal endpoints. The 72 hour E_rC_{50} growth rate endpoint = 0.03278 mg a.s./L, the 72 hour E_rC_{10} growth rate endpoint = 0.01427 mg a.s./L.

Prothioconazole-desthio

Heimbach, 1990b was a 96 hour static algal growth toxicity test carried out on *Scenedesmus subspicatus*. The study was carried out in accordance with OECD 201 and in compliance with GLP. The validity criterion regarding the coefficient of variation of the section-by-section specific growth rates was not met at 72 or 96 hours. This was due to very rapid growth between initial inoculation at the first observations at 24 hours. The other validity criteria were met. Furthermore, analytical verification of concentrations was only carried out at test start, therefore it is not possible to conclude reliable endpoints from this study. Therefore, the endpoints from this study should not be used.

One algal growth toxicity study is available for prothioconazole-desthio (Heimbach, 1990b), this was carried out on *Scenedesmus subspicatus*. It is not possible to conclude reliable endpoints from this study. Therefore, there is no reliable algal toxicity endpoint for prothioconazole-desthio.

There is a data gap for an algal toxicity endpoint for prothioconazole-desthio; however as there are other data available for this degradant that indicate that it would be classificable this does not affect the outcome of the classification of prothioconazole.

Higher aquatic plants

Prothioconazole

Kern *et al*, 2004b was a 7 day static-renewal higher aquatic plant growth toxicity test carried out on *Lemna gibba*. The study was carried out in accordance with OECD 221 and in compliance with GLP. The validity criterion was met. Initial measured concentrations on day 0 and day 5 were within 80-120% of nominal. The measurements from the aged solutions (day 3 and day 7) showed that prothioconazole dissipated from the test system. No test substance was measured in the lowest 4 nominal concentrations in the measured spent solutions. Due to no detectable concentrations being found in the lowest four concentrations after 7 days of exposure, endpoints that rely on the lower concentrations being accurate cannot be relied upon. The growth rate (frond count) E_rC_{50} was found to be higher than the highest tested nominal concentration, for which there are sufficient analytical measurements to calculate a geometric mean measured concentration. There are no EC_{10} or EC_{20} values for growth rate based on frond count or dry weight. The E_rC_{50} > 0.1776 mg a.s./L.

One algal growth toxicity study is available for prothioconazole (Kern *et al*, 2004b), this was carried out on *Lemna gibba*. The E_rC_{50} = 0.1776 mg a.s./L.

Prothioconazole-desthio

Kern *et al*, 2003 was a 7 day static-renewal higher aquatic plant growth toxicity test carried out on *Lemna gibba*. The study was carried out in accordance with OECD 221 and in compliance with GLP. The validity criterion was met. Actual concentrations were not measured in the old solutions at day 3. However, the measured concentrations of fresh and old solutions from day 0 and day 7 showed that prothioconazole is stable

under the conditions of the test for a duration of four days. The intial measured concentrations at day 3 were comparable to those measured at day 0, therefore, it can be concluded that the concentrations in the old solutions at day 3 would have been within the acceptable range. As the intervals between measurements were uneven, the formula in Annex 2 of OECD 23 should have been used to calculate the geometric mean measured concentrations; however this would have very little impact on the resulting concentrations. It is noted that there are no growth rate endpoints based on dry weight. The E_rC_{50} = 0.0809 mg p.m./L and E_rC_{10} = 0.01568 mg p.m./L.

One higher aquatic plant growth toxicity study is available for prothioconazole-desthio (Kern *et al*, 2003)The endpoints are E_rC_{50} = 0.0809 mg p.m./L and E_rC_{10} = 0.01568 mg p.m./L.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No further relevant aquatic effects data are available.

11.6 Long-term aquatic hazard

A summary of all the relevant and reliable information on the long-term aquatic toxicity of prothioconazole and prothioconazole-desthio is presented in Table 44. Studies were conducted according to internationally agreed standard test guidelines and corresponding validity criteria were met. In the following sections, executive summaries of the available studies on prothioconazole and prothioconazole-desthio are provided that give more detailed information on chronic aquatic toxicity.

Studies with prothioconazole were carried with technical material of 97.5 – 98.8 % purity. Studies with prothioconazole-desthio were carried with technical material of 93.7 – 98.8 % purity.

Table 44: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results		Remarks	Reference
			Endpoint	Toxicity (mg a.s./L)		
Fish Early Life-Stage (FELS) Toxicity, OECD Guideline 210, GLP	*Oncorhynchus mykiss*	a.s.	NOEC	0.308 (mm)	97 d, flow-through	Anonymous, 2001l
Fish Early Life-Stage (FELS) Toxicity, OECD Guideline 210, GLP	*Oncorhynchus mykiss*	a.s.	NOEC	0.436 (mm)	91 d, flow-through	Anonymous, 2007b
Daphnia magna, Reproduction, OECD Guideline 211, GLP	*Daphnia magna*	a.s.	NOEC	0.56 (nom)	21 d, Static-renewal	Hendel & Sommer, 2001
Sediment dwelling	*Chironomus riparius*	a.s.	NOEC	9.14 (nom)	28 d, static,	Hendel, 2000a

organisms, Draft OECD Guideline 219 , GLP					spiked water	
Freshwater Algal Growth, Inhibition, OECD Guideline 201, GLP	*Pseudokirchneriella subcapitata*	a.s.	72 hour NOE_rC	0.371 (im^)	96 h, static	Dorgerloh, 2000b
Growth and reproduction of aquatic plants, USEPA Guideline 123-2 (checked against OECD 201), GLP	*Skeletonema costatum*	a.s.	E_rC_{10}	**0.01427 (mm)***	72 h, static	Kern & De Haan, 2004
Fish Early Life-Stage (FELS) Toxicity, OECD Guideline 210, GLP	*Oncorhynchus mykiss*	Prothioconazole-desthio	NOEC	**0.00334 (mm)**	97 d, flow-through	Anonymous, 2002
Daphnia magna, Reproduction, OECD Guideline 211, GLP	*Daphnia magna*	Prothioconazole-desthio	NOEC	0.1 (nom)	21 day semi-static	Dorgerloh and Sommer (2001c)
Mysid, Reproduction, OPPTS 850.1350, GLP	*Americamysis bahia*	Prothioconazole-desthio	NOEC	0.064 (mm)	29 days flow-through	Blankinship *et al* (2003)
Sediment dwelling organisms, Draft OECD Guideline 219, GLP	*Chironomus riparius*	Prothioconazole-desthio	NOEC EC_{10}	2.0 3.77	28 d, static, spiked water	Hendel (2000b)
Sediment dwelling organisms, Draft OECD Guideline 218, GLP	*Chironomus riparius*	Prothioconazole-desthio	NOEC	50 mg/kg	28 d, static, spiked sediment	Picard (2008)

mm: mean measured concentration, nom: nominal concentration

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

* 72 hour endpoint provisional as was estimated by the RMS using 96 hour measured concentrations and the ratio between 96 hour nominal and mean measured concentration endpoints.

11.6.1 Chronic toxicity to fish

Anonymous (2001l) was a 97 day early life stage (ELS) flow-through study carried out with *Oncorhynchus mykiss*. The study was carried out in accordance with OECD 210 and in compliance with GLP. The validity

criterion of hatching success was not met (36% of total eggs, 57% of eggs corrected for fertilisation success compared to a threshold of 75%). All other validity criteria were met. Endpoints were based on mean measured concentrations. Egg hatchability was evaluated on day 40 (post hatch day 3). Hatch data, corrected for natural egg hatchability and fertilisation success, ranged from 47 to 66% in the treatment groups. There was no significant reduction in egg hatchability in any treatment group compared to the pooled controls. Newly hatched fry began to swim up from the bottom of the test chambers on study day 49 (post hatch day 12). Swim-up was first observed on study day 49 (in the solvent control, and the 35.6 and 140 µg a.s./l). First swim up in the 308 µg a.s./l test concentration occurred on day 51. At the highest concentration 553 µg a.s./l swim up was first observed on day 61, when a level of 31% was reported. On day 61 in the controls, more than 90% of the fry had swum-up. Analysis of swim up data between days 61 and 64 was reported when it was significantly reduced in the highest test concentration (553 µg a.s./l). Fry survival was assessed on day 97. There was no difference in fry survival in any of the treatment groups compared with the pooled control (81%). There was no statistically significant difference in fry growth (standard length and dry weight) in any of the treatment groups compared with pooled controls. During the post hatch period the following behavioural and morphological effects were observed sporadically: fish lying on bottom of aquarium, light colouration, reduced paunch, fish lying on their side or on their backs, loss of equilibrium or an open mouth. The study author concludes that there was no evidence that these effects were dose related. The NOEC based on the statistically significant reduction in swim-up observed between study days 61 and 64 = 0.308 mg a.s./L. It was not possible to calculate EC_{10} or EC_{20} values due to a lack of dose response.

Anonymous (2007b) was a 91 day early life stage (ELS) flow-through study carried out with *Oncorhynchus mykiss*. The study was carried out in accordance with OECD 210 and in compliance with GLP. All validity criteria were met. On study day 27, results from the A and B replicates of the 0.50 mg a.s./L nominal treatment showed unexplained high recoveries (0.86 and 0.76 mg a.s./L, relating to 172 and 152% of nominal concentrations, respectively). Following this, the test system was checked to ensure its function and additional samples of this test level were taken on study day 30. These results were 0.39 and 0.56 mg a.s./L, relating to 78 and 112% of the nominal, respectively. The day 30 measurements were used in the calculation of mean measured concentrations in the report rather than the day 27 results for this replicate. There were also diluter system malfunctions on study days 71-72, and 75-76, which affected all groups. Corrective actions were made after each malfunction and several samples were taken to measure test substance concentrations following the malfunctions. Measured concentrations after the first malfunction were 7 to 9 % of nominal and after the second malfunction 32 to 42% of nominal with the exception of the test level with 0.125 mg a.s./L where 2% of nominal was found. After action was taken, the additional measurement indicated increasing levels of test substance. The study report states that based on the turnover volume of the test system and the measurements of test substance concentrations determined after the malfunctions, it was calculated that the test substance was not delivered for about 4 hours during the first malfunction and about 2 hours during the second malfunction. No further details on these calculations were provided. The measurements from days 71, 72, 75 and 76 were excluded from the calculations of mean measured concentrations in the study report. The dossier submitter has recalculated the geometric mean measured concentrations including all the analytical measurement results. All results have been reported in terms of the recalculated mean measured concentrations. Three mortalities occurred in replicate C of the 0.436 mg a.s./L (m.m.) concentration on day 90 (one day before test termination). The oxygen saturation in replicate C on day 90, which was then rectified for the remainder of the study, was very low (32%). However, oxygen saturation was also very low in replicate A (37%) on day 90 and no mortality occurred in this replicate. There was a definite effect on mortality in the higher tested concentration (0.859 mg a.s./L); most of the mortality in this group occurred up to and including day 59. Furthermore, the results from the ELS carried out for the DAR (also on rainbow trout) found there to be no treatment related mortality at the highest concentrations tested, i.e. 0.308 and 0.553 mg a.s./L. It is therefore accepted that the mortalities seen in replicate C on day 90 were not treatment related. All surviving fish at study termination showed normal behaviour with no malformations. No significant reduction in egg hatchability was observed at any test concentration. A statistically significant reduction in fry survival compared to pooled control data was observed in the highest test concentration. A significant difference between pooled control % swim-up and the highest test substance concentration was observed on study days 46, 47, and 48. There were no statistical

difference on dry weight and length compared to the controls. The NOEC based on fry survival and time to swim-up = 0.436 mg a.s./L.

Two early-life stage (ELS) toxicity studies are available for prothioconazole, these were carried out on *Oncorhynchus mykiss*. Anonymous (2001l) was submitted for the first Annex I assessment and Anonymous (2007b) was submitted for the renewal assessment. Anonymous (2007b) was considered completely reliable as the study was conducted to the agreed guideline and to GLP and all the validity criteria were met. It was not possible to calculate EC_{10} or EC_{20} values from either study due to a lack of dose response (the LOEC was the top dose in both studies), therefore only the NOEC values will be considered further. Anonymous (2001l) did not meet the biological validity criterion regarding hatching success; however, the endpoint was based on reduction in swim-up and onset of swim up which are not necessarily affected by hatching success. Furthermore, the endpoint from Anonymous (2007b) is based on swim-up, as well as fry survival, and morphological / behavioural effects. The old and new endpoints are within the same order of magnitude, i.e. 0.308 mg a.s./L compared to 0.436 mg a.s./L, respectively. It is also noted that the old LOEC is greater than the new NOEC. It is not considered appropriate to discount the older endpoint based on the failed validity criterion, therefore, the lowest chronic fish endpoint for classification purposes would be the NOEC from Anonymous (2001l) based on reduction in swim-up and onset of swim up, which is 0.308 mg a.s./L.

Prothioconazole-desthio

Anonymous (2002) was a 96 day early life stage (ELS) flow-through study carried out with *Oncorhynchus mykiss*. The study was carried out in accordance with OECD 210 and in compliance with GLP. The validity criteria were met. Endpoints were based on mean measured concentrations. There were no treatment related effects on time to first hatch, completion of hatching, hatching rate, morphology at hatch and behaviour. There were treatment related effects on post hatch success at 14.1, 27.5 and 53.0 µg p.m./L. Average total length at the end of the study was statistically significant compared to the control at the two highest tested concentrations. The reduction in total length at the end of the study was partly due to a deformation of the head. Some fish from the higher treatment concentrations showed a reduction in snout length which was in general combined with a more rounded head shape. In many cases the lower jaw was not reduced and was therefore longer than the upper jaw. The percentages of deformed individuals (both moderate and severely affected) were 0, 0, 0, 8.5, 10.0, 40.4 and 87.5% in the control and treatment levels 1.90, 3.34, 7.52, 14.1, 27.5 and 53.0 µg p.m./L, respectively. These deformities are considered to be of sufficient concern as to form the basis of the overall NOEC. The NOEC = 0.00334 mg a.s./L.

Anonymous (2004d) was a 9 month fish full life cycle (FFLC) flow-through study carried out with *Pimephales promelas*. The study was carried out in accordance with OPPTS Number 850.1500 and in compliance with GLP. The guideline dose not include validity criteria. Endpoints were based on mean measured concentrations. There were no treatment related effects on hatching success, therefore the NOEC for this parameter was 296 µg p.m./L. No spawning was observed in the highest tested concentration group, i.e. 296 µg p.m./L; therefore, there were treatment related effects on **second generation hatching success**; therefore the NOEC for this parameter was 148 µg p.m./L There were also treatment related effects at 296 µg p.m./L on **larval and juvenile survival and clinical observations of the parental generation**, **juvenile and adult survival and clinical observations of the parental generation**, **mortality and clinical observations of adults**, **first generation growth**, **sex ratio of F0 generation fish** and **observed physical deformities**; therefore the NOEC for these parameters was 148 µg p.m./L.

Five fish in the 148 µg p.m./L treatment group appeared smaller than the other fish and one of the fish had a crooked spine. In the report the NOEC for this parameter was reported as 148 µg p.m./L because the post-hatch survival in replicate B of this group was 100%, whereas post-hatch survival in replicate A was 56 %. However, there is no further explanation as to why replicate A is considered the outlier and not replicate B therefore this NOEC has not been fully justified. The NOEC is considered to be 74 µg p.m./L due to post-hatch survival of the second-generation fish and the observed physical deformities in the 148 µg p.m./L treatment group.

Analysis of the data with Fischer's Exact test showed that **spawning frequency** was significantly reduced in the 19, 37, 148 and 296 µg p.m./L groups in comparison to the pooled controls ($p \leq 0.01$). However, the 74 µg p.m./L group was not significantly different ($p > 0.05$). There was a large difference between the replicates of the 19 µg p.m./L group; the spawning frequency in replicate A is comparable to the frequency in the controls. Also, the spawning frequency in both replicates of the test item groups 37, 74 and 148 µg p.m./L were all higher than the spawning frequency in replicate B of 19 µg p.m./L. There was no obvious reason for the difference between replicates of the 19 µg p.m./L group, e.g. difference in measured abiotic parameters in replicate B. Considering that there was no dose response to support the results of replicate B being within the norm it is considered more likely that this low frequency was an outlier and replicate A was reflective of the normal spawning frequency expected. The rate of spawning in the 74 µg p.m./L treatment group was not statistically significantly different to the control; however as spawning frequency was statistically significantly different in the 37 and 148 µg p.m./L groups it is concluded that a conservative NOEC of 19 µg p.m./L should be concluded for spawning frequency.

Analysis of the data with Williams Test (one-sided, $\alpha = 0.05$) showed that **time to spawning** in the 19, 37 and 74 µg p.m./L groups was not statistically significantly different to the pooled control. Time to spawning in the 148 µg p.m./L group was statistically significantly different to the pooled control results. In addition to this statistically significant difference it is noted that there is no dose response at lower concentrations. Therefore, it is concluded that the NOEC for time to spawning is 74 µg p.m./L.

There appeared to be a biologically significant effect of the solvent control on the **total number of eggs** produced. There appeared to be no dose response until ≥148 µg p.m./L, therefore the proposed NOEC for total egg production is 74 µg p.m./L.

The overall NOEC (based on effects on spawning frequency) = 19 µg p.m./L

One ELS toxicity study is available for prothioconazole-desthio, this was carried out on *Oncorhynchus mykiss* and was submitted for the first Annex I assessment (Anonymous, 2002). This study is considered to be reliable and the endpoints are based on mean measured concentrations. One FFLC study is available for prothioconazole-desthio, this was carried out on *Pimephales promelas* and was submitted for the renewal assessment. This study is considered to be reliable and the endpoints are based on mean measured concentrations. The chronic fish degradant endpoint for classification purposes would be the NOEC based on deformities and is 0.00334 mg p.m./L.

11.6.2 Chronic toxicity to aquatic invertebrates

Prothioconazole

Hendel and Sommer (2001) was a 21 day static-renewal chronic toxicity study carried out with *Daphnia magna*. The study was carried out in accordance with OECD 211 and in compliance with GLP. The validity criteria were met. Analysis was carried out on fresh solutions from days 0, 9 and 19 and not on days 5, 7, 12, 14 and 16. Not all the spent solutions were measured, i.e. only three of the test concentrations were analysed and only on days 2, 12 and 21, i.e. not on days 5, 7, 9, 14, 16 and 19. The analysis showed that the nominal concentrations were achieved in the new solutions ±20% of nominal and that the compound was stable over 2 and 3 days. The information available demonstrates that dissipation between renewals would not have resulted in actual concentrations being less than 80% of nominal if the fresh solutions were always 80-120% of nominal. Not all the fresh solutions were measured, therefore it is not known if all fresh solutions were always 80-120% of nominal, therefore there is uncertainty over the endpoint derived. Endpoints were based on nominal concentrations. There was a time-dependent dose responsive effect on mortality, i.e. the higher the concentration the earlier the mortality was observed. Body length of surviving adults was not affected by the test item. Mean numbers of offspring per adult was affected in test item groups ≥1.0 mg a.s./L. The NOEC based on reduction of offspring = 0.56 mg a.s./L.

One chronic aquatic invertebrate toxicity study is available for prothioconazole (Hendel and Sommer, 2001), this was carried out on *Daphnia magna*. The endpoint is considered reliable and is based on nominal concentrations; this is a NOEC based on a reduction of offspring and is 0.56 mg a.s./L.

Prothioconazole-desthio

Dorgerloh and Sommer (2001c) was a 21 day static-renewal chronic toxicity study carried out with *Daphnia magna*. The study was carried out in accordance with OECD 211 and in compliance with GLP. The validity criteria were met. Analysis was carried out on fresh solutions from days 0, 9 and 19 and not on days 5, 7, 12, 14 and 16. Not all the spent solutions were measured, i.e. only three of the test concentrations were analysed and only on days 2, 12 and 21, i.e. not on days 5, 7, 9, 14, 16 and 19. The analysis showed that the nominal concentrations were achieved in the new solutions ±20% of nominal and that the compound was stable over 2 and 3 days. The information available demonstrates that dissipation between renewals would not have resulted in actual concentrations being less than 80% of nominal if the fresh solutions were always 80-120% of nominal. Not all the fresh solutions were measured, therefore it is not known if all fresh solutions were always 80-120% of nominal, therefore there is uncertainty over the endpoint derived. Endpoints were based on nominal concentrations. Mortality and body length of adults was not affected by the test item at any tested concentrations. Mean numbers of offspring per adult was affected in test item groups ≥0.2 mg p.m./L. The NOEC based on reduction of offspring = 0.1 mg a.s./L.

Blankinship *et al* (2003) was a 29 day flow through chronic toxicity study carried out with *Mysidopsis bahia*. The study was carried out in accordance with OPPTS 850.1350 and in compliance with GLP. The validity criteria were met and the endpoints were based on mean measured concentrations. No test item related effects were observed on survival to pairing, survival after pairing, total body length and mean dry weight. The NOEC for these parameters was 252 µg p.m./L. There was a 32 and 31% reduction in reproduction at the highest two tested concentrations, respectively. The NOEC for reproduction was 64 µg p.m./L, which was also the overall NOEC.

Two chronic aquatic invertebrate toxicity studies are available for prothioconazole-desthio, they were carried out on *Daphnia magna* (Dorgerloh and Sommer, 2001c) and *Americamysis bahia* (Blankinship *et al*, 2003). Dorgerloh and Sommer (2001c) was submitted for the first Annex I assessment, whereas Blankinship *et al* (2003) was submitted for the renewal assessment. Both the endpoints are considered reliable; the *Daphnia magna* endpoint is based on nominal concentrations whereas the *Americamysis bahia* endpoint is based on mean measured concentrations. It was not possible to calculate EC_{10} or EC_{20} values from the *Americamysis bahia* study due to a lack of dose response, therefore the NOEC will be considered further. The lower of the two endpoints is the *Americamysis bahia* NOEC based on reproduction and is 0.064 mg p.m./L.

11.6.3 Chronic toxicity to algae or other aquatic plants

Algae

Prothioconazole

As discussed above in section 11.5.3, the results of Dorgerloh (2000b) and Kern & De Haan (2004) were considered acceptable. As Dorgerloh (2000b) was submitted for the first review of prothioconazole and not as new data for the renewal no EC_{10} endpoints were calculated or requested. A reliable NOE_rC is, however, available from this study, which is 0.371 mg a.s./L.

Kern & De Haan (2004) was submitted as new data for the renewal, therefore an EC_{10} value should have been submitted. As explained above in section 11.5.3, the 72 hour E_rC_{10} growth rate endpoint was estimated based on 96 hour measured concentrations and 72 hour biological results, which is considered to be a conservative approach. The 72 hour E_rC_{10} growth rate endpoint = 0.01427 mg a.s./L.

Prothioconazole-desthio

One algal growth toxicity study is available for prothioconazole-desthio (Heimbach, 1990b), this was carried out on *Scenedesmus subspicatus*. The validity criteria were not met. Furthermore, analytical verification of concentrations was only carried out at test start, therefore it is not possible to conclude reliable endpoints from this study. Therefore, there is no reliable algal toxicity endpoint for prothioconazole-desthio.

There is a data gap for an algal toxicity endpoint for prothioconazole-desthio; however as there are other data available for this degradant that indicate that it would be classificable this does not affect the outcome of the classification of prothioconazole.

Higher aquatic plants

Prothioconazole

As discussed above in section 11.5.3, the results of Kern *et al* (2004b) could not be used to calculate NOEC or EC_{10} endpoints. However, this is not considered to be an issue for classification because algae are significantly more sensitive to prothioconazole than *Lemna*, as evidenced by the significantly lower E_rC_{50} for algae compared to *Lemna.*

Prothioconazole-desthio

As discussed above in section 11.5.3, the results of Kern *et al* (2003) are considered reliable. The E_rC_{10} = 0.01568 mg p.m./L.

11.6.4 Chronic toxicity to other aquatic organisms

Prothioconazole

Hendel (2000a) was 28 day static spiked water chronic toxicity study carried out with *Chironomus riparius* larvae. The study was carried out in accordance with OECD 219 and in compliance with GLP. The validity criteria were met. No effects on emergence rate or development rate at any of the nominal concentrations were observed. Concentrations were measured in the overlying and pore water of three of the nominal concentrations. Four of the nominal concentrations were above the limit of solubility for the active substance. Measurements on day 0 in the 1.14, 9.14 and 57.14 mg a.s./L test groups were 2.47, 9.3 and 5.62 mg a.s/L, respectively, corresponding to 217, 102 and 9.8 % of nominal concentration, respectively. The analysis of the stock solutions showed that the stock solutions were made accurately. The NOEC was set to be 9.14 mg a.s./L because this test solution was achieved at the start of the test, whereas the higher nominal concentrations would not have been achieved, and no effects were observed in this test group. The concentration of the test substance in the sediment was not analytically determined; therefore the dossier submitter followed the advice of OECD 219 and compared the results of Hendel (2000a) to the results of water/sediment study (Brumhard, B. and Oi, M.; 2001, amended 2002) to conclude whether the sediment results from the water/sediment study could be extrapolated to the results of Hendel (2000a). It was concluded that the water results were comparable and therefore the sediment results could be extrapolated. It was shown that prothioconazole partitions into the sediment and therefore *Chironomus riparius* in the toxicity test will have been exposed via the sediment as well as via the water phase. It was concluded that the endpoint is reliable despite the lack of measurement in the sediment in the toxicity study. The NOEC = 9.14 mg a.s./L.

One chronic sediment dwelling invertebrate toxicity study is available for prothioconazole (Hendel, 2000a), this was carried out on *Chironomus riparius*. The endpoint is considered reliable, however it is based on nominal concentrations in the water phase despite the concentrations not being maintained in the water (dissipation into the sediment occurred). Mean measured concentration endpoints are not available from this study. It is clear from the endpoint based on nominal concentrations that this species is not the most sensitive for prothioconazole. The NOEC based on a emergence and development rate and is 9.14 mg a.s./L.

Prothioconazole-desthio

Hendel (2000b) was 28 day static spiked water chronic toxicity study carried out with *Chironomus riparius* larvae. The study was carried out in accordance with OECD 219 and in compliance with GLP. The validity criteria were met. Measurements at day 1 in the 1.00, 8.00 and 32.0 mg p.m./L test groups were 1.08, 7.46 and 12.9 mg p.m./L, respectively, corresponding to 108, 93.3 and 40.3% of nominal concentration,

respectively. The limit of solubility of prothioconazole-desthio is 50 mg/L, i.e. only just above the highest tested concentration of 32 mg/L. The endpoints were based on nominal concentrations. There was a dose responsive effect on percentage emergence of midges ≥4 mg p.m./L, therefore the NOEC for this parameter was 2.0 mg p.m./L. An EC_{10} of 3.77 mg p.m./L was also derived. The analytical results indicated that these concentrations were effectively achieved, therefore these endpoints are considered to be reliable. The concentration of the test substance in the sediment was not analytically determined. However, a spiked sediment study was also available therefore both routes of exposure are covered.

One spiked water chronic sediment dwelling invertebrate toxicity study is available for prothioconazole-desthio (Hendel 2000b), this was carried out on *Chironomus riparius*. The endpoint is considered reliable, however it is based on nominal concentrations in the water phase despite the concentrations not being maintained in the water (dissipation into the sediment occurred). Mean measured concentration endpoints are not available from this study. It is clear from the endpoint based on nominal concentrations that this species is not the most sensitive for prothioconazole-desthio. The EC_{10} (based on emergence) = 3.77 mg p.m./L.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Prothioconazole

Aquatic acute toxicity data on prothioconazole are available for fish, invertebrates, algae and higher aquatic plants. Algae are the most acutely sensitive trophic group with E_rC_{50} values ≤ 1.0 mg/L. The lowest value is 0.03278 mg/L for the marine diatom *Skeletonema costatum*. On this basis, prothioconazole meets criteria from the CLP directive (Annex I, section 4.1, table 4.1.0) for classification in Category Acute 1.

As the lowest acute toxicity endpoint is >0.01 to ≤ 0.1 mg/L the corresponding Acute M-factor is 10.

Prothioconazole-desthio

Aquatic acute toxicity data on prothioconazole-desthio are available for fish, invertebrates and higher aquatic plants. Higher aquatic plants are the most acutely sensitive trophic group with E_rC_{50} values ≤ 1.0 mg/L. The lowest value is 0.0809 mg/L for *Lemna gibba*. On this basis, prothioconazole-desthio would meet the criteria from the CLP directive (Annex I, section 4.1, table 4.1.0) and therefore would be classified as Category Acute 1.

As the lowest acute toxicity endpoint is >0.01 to ≤ 0.1 mg/L the corresponding Acute M-factor would be 10. Whilst this CLH Report does not consider the classification of prothioconazole-desthio itself, thefact that it would likely be classified for its hazard to aquatic life, is used in determining whether parent prothioconazole should be considered ‘rapidly degradable’ according to CLP criteria.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Degradation

As summarised in section 11.1.5, prothioconazole is considered not rapidly degradable according to CLP criteria.

Bioaccumulation

Prothioconazole

The log Pow of 2.0 (at pH 7, 25°C) value is below the CLP log Kow trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP. Other values in buffered solutions were also below this trigger. An experimental bioconcentration study in fish is also available. In the experimental study, whole fish BCF values for prothioconazole were less than 500 indicating a low potential for bioaccumulation. The parent substance was also observed to be extensively metabolised and rapid depuration was observed with depuration DT_{50} values of 0.47-0.8 days (whole fish). On this basis, the substance does not meet CLP criteria as a bioaccumulative substance.

Prothioconazole-desthio

The log Pow of 3.04 value is below the CLP log Kow trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate under CLP. An experimental bioconcentration study in fish is also available. In the experimental study, whole fish BCF values for prothioconazole-desthio were less than 500 indicating a low potential for bioaccumulation. The degrandant was also observed to be extensively metabolised and rapid depuration was observed with depuration DT_{50} values of 0.39-0.47 days (whole fish). On this basis, this major aquatic degradant of prothioconazole would not meet CLP criteria as a bioaccumulative substance.

Chronic toxicity

Prothioconazole

As discussed in sections 11.6.1, 11.6.2 and 11.6.3 there are reliable chronic toxicity endpoints for fish, aquatic invertebrates and algae. The lowest chronic endpoint is for algae, i.e. the 72 hour E_rC_{10} growth rate endpoint = 0.01427 mg a.s./L. As this endpoint is <0.1 mg/L and as prothioconazole is considered not rapidly degradable, the corresponding chronic classification is Chronic Category 1. The relevant Chronic M-factor is 1.

Prothioconazole-desthio

As discussed in sections 11.6.1, 11.6.2 and 11.6.3 there are reliable chronic toxicity endpoints for fish, aquatic invertebrates and higher aquatic plants. The lowest chronic endpoint is for fish, i.e. the NOEC = 0.00334 mg p.m./L. As this endpoint is <0.1 mg/L and as prothioconazole-desthio is considered not rapidly degradable, the corresponding chronic classification would be Chronic Category 1. The relevant Chronic M-factor would be 10. Whilst this CLH Report does not consider the classification of prothioconazole-desthio itself, the fact that it would likely be classified for its hazard to aquatic life, is used in determining whether parent prothioconazole should be considered 'rapidly degradable' according to CLP criteria.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Classification

Prothioconazole

Based on toxicity data and information regarding the degradation, prothioconazole should be classified Category Acute 1 (Acute M Factor 10), Category Chronic 1 (Chronic M Factor 1).

Labelling

Based on classification of acute 1 and chronic 1 the appropriate labelling is as follows:

GHS09 Pictogram	Signal word	Hazard statement
	'Warning'	H410 'Very toxic to aquatic life with long lasting effects'

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 Static, GLP	*Daphnia magna*	48-h EC_{50}	1.3 (nom)**	Heimbach, 1999c
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.

Additional key elements

In this section the key information from the new studies and new data generated during the procedure for renewal of the approval of prothioconazole in accordance with Commission implementing regulation (EU) No 844/2012 is presented (November 2018).

New OECD TG 201 Desmodesmus subspicatus growth inhibition test with degradation product JAU 6476-desthio (Bayer 2018)

Freshwater microalgae *Desmodesmus subspicatus* was exposed to degradation product JAU 6476-desthio (purity 98.3%) for 72 and 96 hours under static exposure conditions to the nominal concentrations of 0.00596, 0.0191, 0.0610, 0.195, 0.625 and 2.00 mg/L. Test was carried out in accordance with OECD TG 201 and US EPA OCSPP 850.4500. The validity criteria were met. The 72-h E_rC_{50} was 2.03 mg/L and 72-h NOE_rC was < 0.00596 mg/L based on nominal concentrations. The 96-hour E_rC_{50} was 0.955 mg/L and the 96-h NOErC was <0.00596 mg/L based on nominal concentrations. RAC notes that study does not affect the acute and chronic classification of the degradation product JAU 6476-desthio.

Statistical re-evaluation of the data

The statistical re-evaluation of the endpoints in the *Pseudokirchneriella subcapitata* growth inhibition study (Dorgerloh, 2000) (D_KCA_8.2.6.1_09) and *Daphnia magna* reproduction study (Hendel and Sommer, 2001) (D_KCA_8.2.5.1_03) with prothioconazole was provided by Industry. The results of the recalculations of the endpoints are as follows:

- Algae test (Dorgerloh, 2000): recalculated study endpoint based on geometric mean measured concentrations is 96-h E_rC_{50} = 2.18 mg/L, which is the same as the endpoint calculated based on initial mean measured concentrations (cited in the Table above).
- Daphnia test (Hendel and Sommer, 2001): recalculated study endpoint based on nominal concentrations is EC_{10} of 0.61 mg/L (reproduction) and the overall NOEC of the study is 0.56 mg/L. In line with the current CLP Guidance, preference is given to the EC_{10} value over the NOEC value. This applies in cases where EC_{10}s are available for the same endpoint. In the view of RAC, the EC_{10} value of 0.61 mg/L (reproduction) over the overall NOEC of 0.56 mg/L for *Daphnia magna* should be selected as the lowest value for this species.

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 $0.01 < E_rC_{50} \leq 0.1$ 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 $0.01 < EC_{10} \leq 0.1$ 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.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

Not considered in this assessment.

12.1.2 Comparison with the CLP criteria

Not considered in this assessment.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not considered in this assessment.

13 ADDITIONAL LABELLING

No additional labelling proposed.

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Author(s)	**Year**	**Title** **Source** ***(where different from company)*** **Company name, Report No., Date, GLP status** ***(where relevant)*****, published or not**	**Owner**
Kern, M. E.; Lam, C. K.	2004	Toxicity of JAU 6476 technical to the freshwater diatom Navicula pelliculosa Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: 200252, Edition Number: M-001064-01-1 EPA MRID No.: 46246109 Date: 2004-03-12 GLP/GEP: yes, unpublished	Bayer CropScience
Kern, M. E.; Roberts, J. A.; de Haan, R. A.	2004	Toxicity of JAU 6476 technical to the blue-green alga Anabaena flos-aquae Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: 200497, Edition Number: M-000348-01-1 EPA MRID No.: 46246103 Date: 2004-02-23 GLP/GEP: yes, unpublished	Bayer CropScience
Nau, M.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20140193.01, Edition Number: M-491727-01-1 Date: 2014-07-11 GLP/GEP: yes, unpublished	Bayer CropScience
Nau, M.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20140193.01, Edition Number: M-491727-01-1 Date: 2014-07-11 GLP/GEP: yes, unpublished	Bayer CropScience
Riegner, K.	1998	Hydrolysis of [phenyl-UL-14C]JAU 6476 in sterile aqueous buffer solutions Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: MR-623/98, Edition Number: M-005117-01-1 Date: 1998-11-16 GLP/GEP: yes, unpublished	Bayer CropScience

Author(s)	**Year**	**Title** **Source** ***(where different from company)*** **Company name, Report No., Date, GLP status** ***(where relevant)*****, published or not**	**Owner**
Schramel, O.	2001	Dissipation of JAU 6476 (250 EC) in soil under field conditions (France, Germany, Great Britain, Italy) Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2152/98, Report includes Trial Nos.: R 812587 R 812595 R 812609 R 812617 R 812625 R 812633 R 815667 R 815675 Edition Number: M-049322-01-1 Date: 2001-03-30 GLP/GEP: yes, unpublished	Bayer CropScience
Wiche, A.; Ziemer, F.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Dissociation constant in water Bayer CropScience, Report No.: PA14/083, Edition Number: M-498202-01-1 Date: 2014-10-02 GLP/GEP: yes, unpublished	Bayer CropScience
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Flammability (solids) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.02, Edition Number: M-525917-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Explosive properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.03, Edition Number: M-525920-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience

Author(s)	**Year**	**Title** **Source** ***(where different from company)*** **Company name, Report No., Date, GLP status** ***(where relevant)*****, published or not**	**Owner**
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Auto-flammability (solids - determination of relative self-ignition temperature) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.04, Edition Number: M-525929-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Oxidizing properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.05, Edition Number: M-525930-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Explosive properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.03, Edition Number: M-525920-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Flammability (solids) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.02, Edition Number: M-525917-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Auto-flammability (solids - determination of relative self-ignition temperature) Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.04, Edition Number: M-525929-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience

Author(s)	**Year**	**Title** **Source** ***(where different from company)*** **Company name, Report No., Date, GLP status** ***(where relevant)*****, published or not**	**Owner**
Winkler, S.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Oxidizing properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: 20150184.05, Edition Number: M-525930-01-1 Date: 2015-06-19 GLP/GEP: yes, unpublished	Bayer CropScience
Ziemer, F.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA15/062, Edition Number: M-528059-01-1 Date: 2015-07-16 GLP/GEP: yes, unpublished	Bayer CropScience
Ziemer, F.	2015	Prothioconazole (JAU6476, AE 1344248), technical substance: Relative density Bayer CropScience, Report No.: PA15/060, Edition Number: M-528054-01-1 Date: 2015-07-16 GLP/GEP: yes, unpublished	Bayer CropScience
Ziemer, F.; Strunk, B.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Physical characteristics colour, physical state and odour Bayer CropScience, Report No.: PA14/079, Edition Number: M-492435-01-1 Date: 2014-07-21 GLP/GEP: yes, unpublished	Bayer CropScience
Ziemer, F.; Strunk, B.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Relative density Bayer CropScience, Report No.: PA14/081, Edition Number: M-493181-01-1 Date: 2014-07-31 GLP/GEP: yes, unpublished	Bayer CropScience
Ziemer, F.; Strunk, B.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Partition coefficients 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC method) Bayer CropScience, Report No.: PA14/071, Edition Number: M-492539-01-1 Date: 2014-07-22 GLP/GEP: yes, unpublished	Bayer CropScience

Author(s)	**Year**	**Title** **Source** ***(where different from company)*** **Company name, Report No., Date, GLP status** ***(where relevant)*****, published or not**	**Owner**
Ziemer, F.; Strunk, B.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Partition coefficients 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC method) Bayer CropScience, Report No.: PA14/071, Edition Number: M-492539-01-1 Date: 2014-07-22 GLP/GEP: yes, unpublished	Bayer CropScience
Ziemer. F.; Strunk, B.	2014	Prothioconazole (JAU6476, AE 1344248), pure substance: Water solubility at pH 4, pH 7 and pH 9 Bayer CropScience, Report No.: PA14/078, Edition Number: M-503425-01-1 Date: 2014-11-20 GLP/GEP: yes, unpublished	Bayer CropScience

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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).

15 ANNEXES

15.1 Annex I: HISTORICAL CONTROL DATA FOR MICROPHTHALMIA IN WISTAR (Wistar, Hsd Cpb:WU) RATS

Year	Study	No. of Foetuses investigated	Foetuses with Microphthalmia No.	%	No. of Litters investigated	Litters with Microphthalmia No.	%
1983	T6007810[++]	218	1	0.46	22	1	4.55
	T2008626	114	1	0.88	12	1	8.33
	7 other studies were conducted in 1983 which showed no microphthalmia in the control group						
1984	T5016710	254	1	0.39	24	1	4.17
	T9016877	173	1	0.58	22	1	4.55
	T8019035	205	4	1.95	22	4	18.18
	6 other studies were conducted in 1984 which showed no microphthalmia in the control group						
1985	T5019339	231	1	0.43	21	1	4.76
	T5019825	122	1	0.82	16	1	8.33
	T0020125[+++]	271	1	0.37	25	1	4
	13 other studies were conducted in 1985 which showed no microphthalmia in the control group						
1986	T5022506	232	1	0.43	24	1	4.17
	T1023484	223	2	0.9	23	2	8.7
	T3024250	253	2	0.79	21	1	4.76
	4 other studies were conducted in 1986 which showed no microphthalmia in the control group						
1987	T6025171[+]	230	2	0.87	24	2	8.33
	T6023777	232	1	0.43	21	1	4.76
	T1027435	185	2	1.08	20	2	10
	4 other studies were conducted in 1987 which showed no microphthalmia in the control group						
1988	T2029650	200	1	0.5	21	1	5
	T1029424	211	2	0.95	24	2	8.33
	T0030368	209	3	1.44	22	2	9.52
	4 other studies were conducted in 1988 which showed no microphthalmia in the control group						
1989	T8030636	228	2	0.88	24	2	8.33
	T5033216	279	1	0.36	24	1	4.17
	4 other studies were conducted in 1989 which showed no microphthalmia in the control group						
1990	T0034599	80	1	1.25	9	1	11.11
1990	T7037368[+]	170	1	0.59	19	1	5.26
	4 other studies were conducted in 1990 which showed no microphthalmia in the control group						
1991	T4040307	262	1	0.38	23	1	4.35
	4 other studies were conducted in 1991 which showed no microphthalmia in the control group						
1992	T9040474	213	2	0.94	22	2	9.1
	T3041008/A[+++]	243	1	0.41	21	1	4.8
	T3041008/V[+++]	263	2	0.76	22	2	9.1

Year	Study	No. of Foetuses investigated	Foetuses with Microphthalmia No.	%	No. of Litters investigated	Litters with Microphthalmia No.	%
	T4040848	231	1	0.43	22	1	4.6
	T9044173	149	2	1.34	13	2	15.4
apr 1993	T4050072	204	1	0.49	20	1	5.0
aug 1993	T7050318	256	5	1.95	23	2	8.7
	2 other studies were conducted in 1993 which showed no microphthalmia in the control group						
1994	T7055548	271	1	0.37	23	1	4.4
	T2058027	315	2	0.63	28	2	7.1
1995	T8058014	281	1	0.4	24	1	4.17
	T2055246	321	5	1.56	28	5	17.86
	1 other study was conducted in 1995 which showed no microphthalmia in the control group						
1996	T1054291	295	1	0.34	26	1	3.85
	T3055247	313	1	0.32	26	1	3.85
	T8054289	255	1	0.39	22	1	4.55
	2 other studies were conducted in 1996 which showed no microphthalmia in the control group including the present study (T2060240)						
1997	T0060860	224	4	1.79	20	4	20.0
	T3060250	217	1	0.46	19	1	5.26
	T8060255	224	1	0.45	18	1	5.56
	1 other study was conducted in 1997 which showed no microphthalmia in the control group						
1998	T7061370	246	1	0.41	21	1	4.76
	T9061390	240	1	0.42	21	1	4.76
	3 other studies were conducted in 1998 which showed no microphthalmia in the control group						
1999	T9061318	256	2	0.78	21	2	9.52
	2 other studies were conducted in 1999 which showed no microphthalmia in the control group						
2000	T5068551	232	0	0.0	20	0	0.0
2001	T1067765	283	1	0.4	22	1	4.5
	T6062800	275	1	0.4	23	1	4.3
	2 other studies were conducted in 2001 which showed no microphthalmia in the control group						
2002	T7062784	269	1	0.4	23	1	4.3
	T6071558	244	4	1.6	20	4	20.0
	T9062786	247	1	0.4	20	1	5.0
	1 other study was conducted in 2002 which showed no microphthalmia in the control group						

[+] dermal application
[++] intravenous application
[+++] inhalation

Control data of 112 studies from 1983 – 2002

15.2 Annex II: HISTORICAL CONTROL DATA FOR RUDIMENTARY SUPERNUMERARY RIBS IN WISTAR (Wistar, Hsd Cpb:WU) RATS

Year	Study	No. of Foetuses investigated	Foetuses with Supernumerary ribs		14th	No. of Litters investigated	Litters with Supernumerary ribs		14th
			No.	%			No.	%	
1990	T6034739+	222	7	5.98		23	4	17.4	
	T9037072	258	14	10.22		23	9	39.1	
	T3037265	63	4	12.12		7	2	28.6	
	T7037368+	170	16	17.98		19	7	36.8	
	T6039518	248	16	12.31		24	11	45.8	
1991	T3038066	236	14	11.3		22	9	40.9	
	T4039958	137	21	15.3		14	8	57.1	
	T6040039	220	4	3.5		20	3	15.0	
	T4040307	262	12	8.8		23	8	34.8	
	T3040711	134	9	13.0		14	5	35.7	
1992	T9040474	113	19	16.8		22	11	50	
	T3041008/A^{+++}	126	32	24.4		21	12	57	
	T3041008/V^{+++}	138	26	19.8		22	12	55	
	T4040848	120	14	11.7		12	10	45.5	
	T9044173	77	7	9.1		13	3	23	
apr 1993	T4050072	108	9	8.3		20	8	40.0	
	T4050072 LD	n.a.	n.a.	11.3		n.a.	n.a.	38.1	
aug 1993	T7050318	131	6	4.6		23	4	17.4	
nov 1993	T1050105	123	15	12.2		24	12	50.0	
1994	T7055548	141	5	3.5		23	5	21.7	
	T7055548 LD	n.a.	n.a.	6.9		n.a.	n.a.	25.0	
	T2058027	164	5	3.0		28	4	14.3	
	T2058027 LD	n.a.	n.a.	3.3		n.a.	n.a.	17.4	
1995	T8058014	147	4	2.7		24	4	16.7	
	T2055246	143	2	1.4		24	2	8.3	
	T2055246 LD	n.a.	n.a.	4.7		n.a.	n.a.	14.8	
	T1055245	n.a.	n.a.	3.2		n.a.	n.a.	15.4	
	T1055245 LD	n.a.	n.a.	7.0		n.a.	n.a.	32.0	
feb 1996	**T2060240** [a]	**152**	**1**	**0.7**		**26**	**1**	**3.8**	
1996	T4060260	142	3	2.1		25	3	12.0	
	T1054291	152	3	2.0		26	2	7.7	
	T1054291 LD	n.a.	n.a.	3.3		n.a.	n.a.	20.8	
	T3055247	163	3	1.8		26	3	11.5	
	T3055247 LD	n.a.	n.a.	3.8		n.a.	n.a.	16.0	
	T8054289	133	2	1.5		22	1	4.5	
	T8054289 LD	n.a.	n.a.	4.5		n.a.	n.a.	26.9	
1997	T2055255	147	4	2.7		23	3	13.0	
	T2055255 LD	n.a.	n.a.	0.8		n.a.	n.a.	5.3	
	T0060860	116	4	3.4		20	3	15.0	
	T0060860 LD	n.a.	n.a.	5.1		n.a.	n.a.	27.3	

Year	Study	No. of Foetuses investigated	Foetuses with Supernumerary 14th ribs		No. of Litters investigated	Litters with Supernumerary 14th ribs	
			No.	%		No.	%
	T3060250	113	3	2.7	19	2	10.5
	T3060250 LD	n.a.	n.a.	2.5	n.a.	n.a.	11.8
	T8060255	117	6	5.1	18	3	16.7
	T8060255 LD	n.a.	n.a.	2.4	n.a.	n.a.	14.3
1998	T2061366	144	8	5.6	24	4	16.7
	T2061366 LD	n.a.	n.a.	1.9	n.a.	n.a.	12.0
	T7061370	128	2	1.6	21	2	9.5
	T7061370 LD	n.a.	n.a.	6.1	n.a.	n.a.	21.7
	T8061380	136	4	2.9	23	3	13.0
	T8061380 LD	n.a.	n.a.	0.9	n.a.	n.a.	5.0
	T2061375	120	0	0.0	21	0	0.0
	T2061375 LD	n.a.	n.a.	4.6	n.a.	n.a.	17.6
	T9061390	125	2	1.6	21	2	9.5
1999	T9067880	128	12	9.4	21	7	33.3
	T9067880 LD	n.a.	n.a.	0.8	n.a.	n.a.	4.5
	T2061311	126	6	4.8	22	4	18.2
	T2061311 LD	n.a.	n.a.	1.5	n.a.	n.a.	8.7
	T9061318	133	8	6	21	4	19.0
	T9061318 LD	n.a.	n.a.	2.2	n.a.	n.a.	13.6
	T0061319 [b]	-	-	-	-	-	-
	T0061319 LD	n.a.	n.a.	7.8	n.a.	n.a.	28.6
2000	T5068551	123	16	13	20	6	30
2001	T5067750	n.a.	n.a.	6.6	n.a.	n.a.	30
	T1067765	n.a.	n.a.	10	n.a.	n.a.	45.5
	T8068563	n.a.	n.a.	8	n.a.	n.a.	33.3
	T6062800	n.a.	n.a.	5	n.a.	n.a.	30.4
2002	T3068568	n.a.	n.a.	12.8	n.a.	n.a.	50
	T7062784	n.a.	n.a.	9.2	n.a.	n.a.	43.5
	T6071558	n.a.	n.a.	11.1	n.a.	n.a.	45
	T9062786	n.a.	n.a.	15	n.a.	n.a.	50
	T3063590	n.a.	n.a.	11.6	n.a.	n.a.	42.1
	T5063600	n.a.	n.a.	16.3	n.a.	n.a.	65
2003	T7063008	n.a.	n.a.	13.6	n.a.	n.a.	52.2
	T7062955	n.a.	n.a.	12.4	n.a.	n.a.	57.1

LD unaffected low dose group of studies from 1993-1999 (M-576707-01-1). Low dose groups were considered unaffected if there was no effect on the incidence of supernumerary 14th ribs up to and including the highest dose tested.

[a] present study

[b] same control group as study T9061318

n.a. not available

Control data of 53 studies from 1990 – 2003

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON PROTHIOCONAZOLE (ISO); 2-[2-(1-CHLOROCYCLOPROPYL)-3-(2-CHLOROPHENYL)-2-HYDROXYPROPYL]-2,4-DIHYDRO-3H-1,2,4-TRIAZOLE-3-THIONE

15.3 Annex III: HISTORICAL CONTROL DATA FOR MICROPHTHALMIA / ANOPHTHALMIA IN THE WISTAR HANOVER RAT

TABLE 3

Historical Control for the
Incidence of Microphthalmia/Anophthalmia[a]
in the Wistar Hanover Rat [Crl:WI(HAN)]

Laboratory I.D. No.	In-Life Exposure	Breeder	Diet	Housing	Conducting Laboratory	Unit	Microphthalmia	Anophthalmia
01-T12-EW	07/01-01/02 (GD 6-19)	Charles River Raleigh, NC	Purina Mills Rodent Lab Chow 5002 Meal	Separately in suspended polycarbonate cages	Bayer CropScience LP Toxicology Stilwell, KS	Litter - Fetal -	0/26 (0%) 0/143 (0%)	0/26 (0%) 0/143 (0%)
99-T12-CL	06/99-12/99 (GD 6-17)	Charles River Raleigh, NC	Purina Mills Rodent Lab Chow 5002 Meal	Separately in suspended polycarbonate cages	Bayer CropScience LP Toxicology Stilwell, KS	Litter - Fetal -	0/26 (0%) 0/124 (0%)	0/26 (0%) 0/124 (0%)
98-622-QZ (JAU 6476)	08/98-12/98 (GD 0-19)	Charles River Raleigh, NC Chow 5001-4	Purina Mills Rodent Lab polycarbonate cages	Separately in suspended Stilwell, KS	Bayer CropScience LP Toxicology	Litter - Fetal -	0/23 (0%) 0/106 (0%)	0/23 (0%) 0/106 (0%)
98-612-PL	03/98-9/98 (GD 0-19)	Charles River Raleigh, NC	Purina Mills Rodent Lab Chow 5001-4	Separately in suspended polycarbonate cages	Bayer CropScience LP Toxicology Stilwell, KS	Litter - Fetal -	0/22 (0%) 0/98 (0%)	0/22 (0%) 0/98 (0%)

[a] Microphthalmia or anophthalmia was not observed in any compound-treated group for any of the above stated studies.