Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

propiconazole (ISO); (2*RS*,4*RS*;2*RS*,4*SR*)-1-{[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-1*H*-1,2,4-triazole

EC Number: 262-104-4
CAS Number: 60207-90-1

CLH-O-0000001412-86-139/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

9 December 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Propiconazole

EC Number: **262-104-4**

CAS Number: **60207-90-1**

Index Number: **613-205-00-0**

Contact details for dossier submitter: **Finnish Competent Authority
Finnish Safety and Chemicals Agency (Tukes)
Finland**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Propiconazole
EC number:	262-104-4
CAS number:	60207-90-1
Annex VI Index number:	613-205-00-0
Degree of purity:	≥ 94.0 % (w/w)
Impurities:	Confidential; No impurity is considered relevant for the classification of propiconazole.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 4 *; H302 Skin Sens. 1; H317 Aquatic Acute 1; H400 Aquatic Chronic 1; H410
Current proposal for consideration by RAC	Removal of asterisk (*) from Acute Tox. 4 H302 Skin Sens. 1 H317 Repr. 2; H361d Aquatic Acute 1 H400: Addition of M-factor of 1 Aquatic Chronic 1 H410: Addition of M-factor of 1

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H302 Skin Sens. 1; H317 Repr. 2; H361d Aquatic Acute 1; H400, M-fator of 1 Aquatic Chronic 1; H410, M-factor of 1

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	Inconclusive: test result negative according to the requirements under DSD but no test carried out under CLP
2.2.	Flammable gases	-	-	-	Data conclusive but not sufficient for classification
2.3.	Flammable aerosols	-	-	-	Data conclusive but not sufficient for classification
2.4.	Oxidising gases	-	-	-	Data conclusive but not sufficient for classification
2.5.	Gases under pressure	-	-	-	Data conclusive but not sufficient for classification
2.6.	Flammable liquids	-	-	-	Data conclusive but not sufficient for classification
2.7.	Flammable solids	-	-	-	Data conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	-	-	-	Data lacking
2.9.	Pyrophoric liquids	-	-	-	Data conclusive but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	Data conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	-	-	-	Data conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Data conclusive but not sufficient for classification
2.13.	Oxidising liquids	-	-	-	Data conclusive but not sufficient for classification
2.14.	Oxidising solids	-	-	-	Data conclusive but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.15.	Organic peroxides	-	-	-	Data conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	-	-	-	Data conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	**Acute Tox. 4 H302**	-	**Acute Tox. 4* H302**	-
	Acute toxicity - dermal	-	-	-	Data conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Data conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-	-	-	Data conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Data lacking
3.4.	Skin sensitisation	**Skin Sens. 1 H317**	-	**Skin Sens. 1 H317**	-
3.5.	Germ cell mutagenicity	-	-	-	Data conclusive but not sufficient for classification
3.6.	Carcinogenicity	-	-	-	Data conclusive but not sufficient for classification
3.7.	Reproductive toxicity	**Repr. 2; H361d**	-	-	-
3.8.	Specific target organ toxicity –single exposure	-	-	-	Data conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure		-	-	conclusive but not sufficient for classification-
3.10.	Aspiration hazard	-	-	-	Data conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1 H400** **Aquatic Chronic 1 H410**	**M-factor of 1** **M-factor of 1**	**Aquatic Acute 1 H400** **Aquatic Chronic 1 H410**	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
5.1.	Hazardous to the ozone layer	-	-	-	Data conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning
Pictograms: GHS07, GHS08, GHS09
Hazard statements: H302, H317. H361d, H410
Precautionary statements: No precautionary statements are proposed since they are not included in Annex VI of Regulation (EC) No 1272/2008

Proposed notes assigned to an entry: None proposed

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The hazard classification of propiconazole according to Dangerous Substances Directive (DSD) 67/548/EEC was first agreed in the November 1999 meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances (Pesticides) for environment (ECBI/07/00) and in the February 2002 for health (ECBI/62/02). The agreed classification was Xn; R22, R43, N; R50-53 which was included in Annex I of DSD in the 29th ATP (2004/73/EC) and translated to the CLP Classification Acute Tox. 4*: H302, Skin Sens. 1: H317, Aquatic Acute 1: H400 and Aquatic Chronic 1: H410 in Annex VI of CLP. A classification proposal was submitted to ECHA by the Finnish CA in 2010 to add an M-factor to the aquatic classification but the proposal was withdrawn in 2011 as a result of receiving new data on aquatic toxicity.

2.2 Short summary of the scientific justification for the CLH proposal

Propiconazole is being evaluated as an existing active substance by Finland in both the AIR-3 review program covered by legislation on placing plant protection products on the market and in Risk Assessment review program covered by legislation on making available on the market and use of biocidal products. This classification proposal is based on the Draft Assessment Report (DAR; Finland 1998), and Addendums (Finland, 2002), draft Renewal Assessment Report (dRAR, Finland 2015), Competent Authority Report, Document IIA (CAR; Finland 2015), and on scientific peer-reviewed open literature.

The existing classification of propiconazole for acute oral toxicity is Acute Tox 4*; H302. The oral LD_{50} was 550 mg/kg bw, meeting the criteria for classification under CLP as Acute Tox 4; H302. The asterisk (*) indicating minimum classification is no longer necessary since the data confirms the classification. No classification is warranted for acute toxicity via the dermal or inhalation routes with the dermal LD_{50} being >4000 mg/kg bw and the inhalation LC_{50} >5800 mg/m^3.

Propiconazole is currently classified as Skin Sens. 1; H317. The presented human and animal skin sensitation data do not allow classification into sub-categories 1A or 1B, therefore the existing classification should be retained.

Repeated dose effects were reported in the liver in rats and mice, and in the spleen in the rat. In the rat increased liver weight was observed but there was only limited evidence of changes in the liver enzymes or in histopathology. In the mouse the liver weight effects were associated with changes in enzyme levels, serum cholesterol and histopathology. Liver necrosis was seen in the sub-chronic studies at 500 ppm (i.e. 71 mg/kg bw/day in 13 week; 65 mg/kg bw/day in 17 week) and higher doses. In 18-months carcinogenicity study in mice slightly increased incidence of hepatocellular necrosis was observed at 850 ppm (108 mg/kg bw/day) after 9 weeks treatment, yet after 52 weeks or 18 months similar findings were not reported. In a two-year carcinogenicity study significant increase in hepatocellular necrosis was observed in males of the highest dose group (2500 ppm, 344 mg/kg bw/day) after one year, however at terminal sacrifice there was no difference between the treated and control groups. The dose levels of 71 and 65 mg/kg/day fall within the Category 2 range for STOT-RE classification, however, the severity of hepatocellular necrosis observed was only very slight to moderate and the necrosis appeared to subside with time. These findings are not considered to indicate toxicologically significant functional disturbance or morphological change in the liver and therefore no classification is proposed

There is one carcinogenicity study available in the rat and two carcinogenicity studies available in the mouse. In the rat, there was no evidence of treatment related increase in tumour incidences. Following dietary administration for up to 2 years, high doses of propiconazole resulted in an increased incidence of liver tumours in male CD-1 mice. Incidence of adenomas was statistically significantly increased and was slightly above the contemporary historic control range at 108 mg/kg bw/day and 344 mg/kg bwday. Incidence of liver carcinomas was also significantly increased at 344 mg/kg bw/day. However, this dose level clearly exceeded maximum tolerated dose and the increased incidence of carcinomas was only observed after two years of propiconazole treatment. In addition, the increased incidence of carcinomas was primarily due to an increase in the number of well differentiated hepatocellular carcinomas and there was no significant difference in the morphologic appearance or biological behaviour of the carcinomas observed in the control as compared to the treated groups. In female mice there were no significant differences in liver tumour incidences. Thus, propiconazole promoted formation of spontaneously occurring tumours in one species (mice), in one tissue (liver), and in one sex (male). The above described factors (possibility of confounding effect of excessive toxicity, reduced tumour latency, spontaneous tumours only at high doses) decrease the level of concern for human carcinogenicity. Therefore it is considered that the data available do not support a classification for carcinogenicity for propiconazole.

Propiconazole had no adverse effects on fertility, mating or gestation in a rat two generation reproduction study. Exposure to the highest dose (215 mg/kg bw/day in males, 243 mg/kg bw/day in females) caused significantly reduced pup weights over lactation period during the first generation, and reductions in litter size, in number of viable pups delivered and in pup survival over lactation period and increased number of runt pups during the second generation. However, since signs of liver toxicity (hepatocyte hypertrophy and vacuolization, reduced body weights and food consumption) were observed in both parental animals and the progeny at this dose, it seems plausible that these effects are secondary consequences of systemic toxicity rather than specific effects of propiconazole on reproduction.

The potential of propiconazole to induce prenatal developmental toxicity was investigated in two rat studies and in one rabbit study**.** In rat developmental toxicity study cleft palates occurred in 1/302 (0.33%) pups at an intermediate dose (90 mg/kg bw/day) and in 2/285 pups (incidence 0.7%) of different litters at the high dose (360/300 mg/kg bw/day). In a supplementary developmental toxicity study cleft palate was also observed, but with lower incidence (2/2064 fetuses from 2/158 litters, incidence 0.097%). Cleft palate had not been seen previously in the performing laboratory and the observed incidences are also above the historical control data of other laboratories (4/25522, 0.016%). In both studies marked maternal toxicity was evident at the high dose, but maternal toxicity was only moderate at the intermediate dose. Thus, although the incidences of cleft palates in rats were low it cannot be ruled out that the effect is treatment-related and classification of propiconazole for developmental effects should therefore be considered.

In the rat, significantly increased incidence of skeletal variations (rudimentary ribs and non-ossified sternebrae) at the intermediate (90 mg/kg bw/day) and high doses (360/300 mg/kg bw/day), and increased incidence of urinary tract variations at high dose were also observed. These variations occurred in association with maternal toxicity, and they may represent a delay in development secondary to maternal toxicity. In the rabbit administration of 400 mg/kg bw/day (high dose) propiconazole caused marked maternal toxicity, increased incidences of resorptions, abortions and early deliveries and an increased incidence of fully formed 13th rib in fetuses. The increased incidence of fully formed 13th rib is a variation and occurred only in association with marked maternal toxicity. However, it is not possible to conclude whether increased incidences of resorptions, abortions and early deliveries in rabbit were secondary to maternal toxicity. Thus, these effects cause additional concern for developmental toxicity of propiconazole. In conclusion, primarily based on low

incidences of cleft palates in the two rat studies, propiconazole is proposed to be classified for developmental effects as Repr. 2; H361d. No classification for fertility effects is proposed.

Propiconazole is considered hydrolytically stable under environmentally relevant conditions. A ready biodegradation test resulted in 3% degradation (based on theoretical carbon dioxide) at day 28. On this basis, it is concluded that propiconazole is not readily biodegradable. Mineralisation was only a minor element of dissipation of propiconazole in aquatic water/sediment systems. On this basis propiconazole is not considered to undergo rapid ultimate degradation. Consequently propiconazole is considered not rapidly degradable for the purposes of classification.

The log Pow of 3.72 is lower than the trigger value of 4 for Regulation EC 1272/2008.

In water/sediment study up to eight minor metabolites were detected with maximum concentrations not exceeding 5% of the applied radioactivity for any of them. In the biodegradation test for surface water one major metabolite (1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol) was found, reaching a maximum level of 41.8% of applied radioactivity in low dose test vessels (10 µg/L). However, that metabolite has been demonstrated to be less toxic than propiconazole and therefore this classification proposal is based only on propiconazole ecotoxicity.

A full set of valid acute fish, invertebrate and algae/aquatic plant data are available for propiconazole. Based on the available acute toxicity data for fish, aquatic invertebrates and algae, the lowest acute toxicity value, is an EC_{50} of 0.51 mg/l for *Americamysis bahia* which is between 0.1 and ≤1 mg/L leading to an M factor of 1. Based on chronic aquatic toxicity data of fish, aquatic invertebrates and chronic NOEC for algae, the lowest chronic toxicity value, is a NOEC of 0.068 mg/l for *Cyprinodon varieagatus* which is between 0.01 and ≤ 0.1 mg/L leading to an M factor of 1 for this not rapidly degradable substance. Consequently the classification as Aquatic Acute 1, M-factor 1 and Aquatic Chronic 1, M-factor 1 is applicable.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification		Labelling		Specific Conc. Limits, M-factors
Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	
Acute Tox. 4 * Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H400 H410	GHS07 GHS09 Wng	H302 H317 H410	

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Classification	Labelling	Concentration limits
Xn; R22	Xn; N	-

R43 N; R50-53	R: 22-43-50/53 S: (2-)-36/37-46-60-61	

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification and labelling of propiconazole is according to its current entry in Annex VI to CLP.

RAC general comment

Propiconazole is an active substance in the meaning of EU Regulation 1107/2009 concerning the placing of plant protection products on the market and in the meaning of EU regulation 528/2012 concerning the making available on the market and use of biocidal products. Propiconazole has the following current entry in Annex VI of CLP regulation:
Acute Toxicity 4* (H302); Skin Sensitisation 1 (H317); Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

The Dossier Submitter (DS) proposed the following harmonised classification, based on previous European assessments[1] and on open, peer-reviewed scientific literature:
Acute Toxicity 4 (H302); Skin Sensitisation 1 (H317); Reproductive Toxicity 2 (H361d); Aquatic Acute 1 (H400, M-factor of 1) and Aquatic Chronic 1 (H410, M-factor of 1).

The DS reviewed only the hazards acute toxicity, skin sensitisation, STOT RE, carcinogenicity and reprotoxicity in the CLH dossier but also included information about germ cell mutagenicity as supporting information for the carcinogenicity endpoint. Nonetheless, during the Public Consultation (PC) some comments addressed to germ cell mutagenicity, skin and eye corrosion/irritation were also received.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Propiconazole is an active substance in the meaning of Directive 91/414/EEC (subsequently replaced by EU Regulation 1107/2009) concerning the placing of plant protection products on the market and in the meaning of Directive 98/8/EEC (subsequently replaced by EU Regulation 528/2012) concerning the making available on the market and use of biocidal products, therefore subject to harmonised classification and labelling in accordance with Article 36(2) of the CLP Regulation.

[1] a) Draft Assessment Report (DAR; Finland 1998);
b) DAR Addenda (Finland, 2002);
c) Draft Renewal Assessment Report (dRAR, Finland 2015), and
d) Competent Authority Report on the Document IIA (CAR; Finland 2015).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

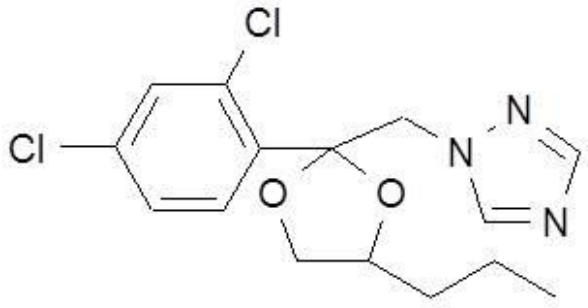
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	262-104-4
EC name:	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole
CAS number (EC inventory):	60207-90-1
CAS number:	60207-90-1
CAS name:	1H-1,2,4-Triazole, 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-
IUPAC name:	(±)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole
CLP Annex VI Index number:	613-205-00-0
Molecular formula:	$C_{15}H_{17}Cl_2N_3O_2$
Molecular weight range:	342.2 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Propiconazole	≥ 94.0 % (w/w)	-	Minimum purity of the active substance as manufactured

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential	-	-	-

The impurities have been taken into consideration in the classification of this substance. Details on the impurities are considered to be confidential and further information is provided in the IUCLID file and flagged confidential.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				-

1.2.1 Composition of test material

The purity of propiconazole tested in the studies ranged from 89.7 to 99.8%. Information on the actual purity used is provided in the relevant tables of this report. The tested material in all cases is considered to be equivalent to and representative of that specified above.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	a) 98.8% pure: clear, colourless viscous liquid. 92.2% technical: yellowish viscous liquid b) 97.16% pure: yellowis viscous liquid c) 99.8% pure: pale yellow amorphous wax like semisolid . tech: yellowish viscous oil	a) Das, 1994 and Das 1993c b) Campbell, 1996 c) Werle, 1994	Visual
Melting/freezing point	98.8% pure: -23°C galss transition temperature	Geoffrey, 1994	EEC A.1 Method EEC A.1 differential scanning calorimetry
Boiling point	a) 92.2% technical: > 250°C at 101.325 kPa; 99.1% pure: 120°C at 1.9 Pa, calculated from vapour pressure curve b) 97.16% technical: 189-195°C at 1.0-1.4 mbar c) 99.8% pure: decomposes above 300°C	a) Das, 1993a b) Campbell, 1996 c) Werle, 1994	EEC A.2 a) Siwoloboff method b) vacuum distillation method (purified active substance not used) c) Siwoloboff method.
Relative density	a) 92.2% technical: 1.289 g/cm^3 at 20°C b) 97.16% technical: 1.2793 g/ml at 20°C c) 99.8% pure: 1.232 g/ml at 20°C	a) Das, 1993b b) Campbell, 1996 c) Werle 1994	EEC A.3 a) oscillating density meter b) pycnometer method c) pycnometer method
Vapour pressure	a) 99.1% pure: 5.6 . 10^{-5} Pa at 25°C, extrapolated b) Technical: 1.25 . 10^{-4} Pa at 25°C extrapolated c) 99.8% pure: 2.1 . 10^{-4} Pa at 20°C and 3.6 . 10^{-4} at 25°C	a) Rordorf, 1988 b) Campbell, 1996 c) Werle, 1994	EEC A.4 a) gas saturation method b) balance method c) gas saturation method

Property	Value	Reference	Comment (e.g. measured or estimated)
Surface tension	a) (92.4 % technical) filtrates of 10.0 g/l suspensions: σ = 47.5 mN/m at 20 °C filtrates of 1.0 g/l suspensions: σ = 59.0 mN/m at 20 °C b) (97.16 % technical) saturated solution: σ = 54.5 mN/m at 20 °C c) (99.8 % pure) 90 % saturated solution: 48.7 mN/m at 20 °C	a) Ryser 1994 b) Campbell 1996 c) Werle 1994	EEC A.5 OECD 115 a) Wilhelmy plate method (ISO 304) Influence of impurities observed. The method is comparable to ring method. b) Ring method c) Ring method
Water solubility	a) 99.1% pure: 100mg/L at 20°C and pH 6.9 (pure water) b) 97.16% technical: 177 mg/L at 26°C (pure water) and 153 mg/L at 26°C and pH 7 (buffer) c) 99.8% pure: 150 mg/L at 20°C and pH 5.2 (pure water)	a) Jäkel, 1987a b) Campbell, 1996 c) Werle, 1994	EEC A.6 a, b and c all used flask method b) purified active substance not used; pH of water not given
Partition coefficient n-octanol/water	a) 99.1% pure: log Pow =3.72 at 25°C and pH 6.6 b) 97.16% technical: log Pow = 3.8 c) 99.8% pure: log Pow = 3.51 at 20°C and pH 4.7	a) Jäkel, 1987b b) Campbell, 1996 c) Werle, 1994	EEC A.8 a) Shake flask method b) HPLC method. Temperature and pH not given c) shake flask nethod
Flash point	a) 92.4% technical: 200°C (1013 mbar) b) 97.16% technical: no flash point, at ca 300°C combustion c) not applicable, semisolid consistency	a) Scűrch, 1994b b) Campbell, 1996 c) Werle, 1994	EEC A.9 Pnsky-Martens
Flammability	a) 92.4% technical: self- ignition temperature 430°C c) 99.8% pure: no ignition	a) Scűrch, 1994a c) Werle, 1994	a) EEC A.15 c) EEC A.10
Explosive properties	a) 92.4% technical: not explosive c) 99.8% pure: not explosive	a) Scűrch, 1994c c) Werle, 1994	EEC A.14 Steel sleeve test (thermal sensitivity) Falling hammer (mechanical sensitivity)

Property	Value	Reference	Comment (e.g. measured or estimated)
Self-ignition temperature	a) 92.4% technical: self- ignition temperature 430ºC c) 99.8% pure: no ignition	a) Scűrch, 1994a c) Werle, 1994	a) EEC A.15 c) EEC A.10
Oxidising properties	Not oxidising substance	Angly, 2000	92.4% technical. Method Section 34 UN 1999
Granulometry	Not relevant		
Stability in organic solvents and identity of relevant degradation products	a) (92.2 % and 92.4 % technical) acetone: completely miscible at 25 °C dichloromethane: completely miscible at 25 °C ethanol: completely miscible at 25 °C ethyl acetate: completely miscible at 25 °C n-hexane: 47 g/l at 25 °C n-octanol: completely miscible at 25 °C toluene: completely miscible at 25 °C b) (97.16 % technical) acetone: completely miscible at ambient dichloromethane: completely miscible at ambient methanol: completely miscible at ambient ethyl acetate: completely miscible at ambient n-hexane: 100 g/l at 22-25 °C toluene: completely miscible at ambient c) (99.8 % pure) acetone: completely miscible at 20 °C dichloromethane: completely miscible at 20 °C methanol: completely miscible at 20 °C ethyl acetate: completely miscible at 20 °C n-heptane: 1.585 g/l at 20 °C xylene: completely miscible at 20 °C	a) Stulz 1994a b) Campbell 1996 c) Werle 1994	a) solvent saturation method b) OECD 105 and 116, and CIPAC MT 157, solvent saturation method c) EEC A.6, solvent saturation method
Dissociation constant	a) 99.1% pure: pK_a = 1.09 at 20°C. b) 97.16% technical: not determinable c) 99.8% pure. The substance does not have acid or alkaline properties	a) Stulz, 1994 b) Campbell, 1996 c) Werle, 1994	OECD 112 a) Spectrophotometric titration b) titration method
Viscosity	At 20°C : 69.630 +/- 0.379 Pa s, 0.10 < D < 10.0 sec^{-1} At 40°C : 3.509 +/- 0.39 Pa s, 0.10 < D < 10.0 sec^{-1} (92.4 %)	CAR, Doc IIA, A.3.14	Rotational viscometer method/OECD No. 114

2 MANUFACTURE AND USES

2.1 Manufacture

The active substance is manufactured in and outside of the EU.

2.2 Identified uses

Propiconazole is used as a fungicide in the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification is proposed based on the evaluated data.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, distribution, metabolism and elimination)

Following studies and references are taken from the Draft Assessment Report (DAR) of propiconazole.

4.1.1 Non-human information

Table 9: Toxicokinetics studies

Route **Guideline** **GLP**	**Species** **Strain** **Sex** **No of animals**	**Administration**	**Reference**
Oral (gavage) Not a guideline study non-GLP	Rat Sprague Dawley TifRAIf (SPF) males and females 2 animals/sex/dose	Single oral dose of 0.5 or 25 mg/ bw of Triazole-^{14}C-propiconazole per animal in 1 ml vehicle/solvent.	DAR IIA 5.1.1/01 Acceptable
Intravenous and oral U.S. FIFRA Subdiv. F§85-1 GLP	Rat Sprague Dawley Crl:CD (SD) BR males and females 5 animals/sex/dose	Single i.v. or oral dose of 0.5 mg/kg bw Phenyl-^{14}C-propiconazole, 0.5 mg/kg bw orally after 14 day pre-treatment, 50 mg/kg bw orally, all doses in 1 ml vehicle.	DAR IIA 5.1.1/02 Acceptable
Oral (gavage) Japan (II) Testing Guidelines of Toxicology Studies GLP	Rat Sprague Dawley TifRAIf (SPF) males 3-6 animals/sex/dose	Single oral dose of 4-5 µCi phenyl-^{14}C-propiconazole (~0.5 mg/kg bw) in 1 ml vehicle.	DAR IIA 5.1.1/03 Acceptable
Dermal Not specified guideline non-GLP	Rat Sprague Dawley males and females 4 animals/sex/dose	Single dermal dose of 1 mg/kg bw and 10 mg/kg bw of Triazole-^{14}C-propiconazole in 10-20 µl solvent.	DAR IIA 5.1.1/04 Acceptable

Route **Guideline** **GLP**	**Species** **Strain** **Sex** **No of animals**	**Administration**	**Reference**
Dermal Not a guideline study non-GLP	Rat Sprague Dawley males 24 animals/dose (4 animals/sacrifice group/dose)	Daily dermal dose during 10 or 24 h:s, followed by a 72 h depletion phase. Triazole-^{14}C-propiconazole 0.01, 0.1 and 1 mg/cm^2 (0.1, 1.0 and 10 mg/rat) dermally to the shaved back skin (10 cm^2), covered but not occluded, in 50-100 µl vehicle.	DAR IIA 5.1.1/05 Acceptable
Oral (feed) Guideline not specified non-GLP	Mouse CD-1 Rat Sprague Dawley TifRAIf (SPF) 21 day pretreatment + radiolabel: 5 mice/sex/dose. Single radiolabel only: 3 female mice and 2 male rats	Unlabelled a.s.: 5, 100 and 2500 ppm/day for 21 days Labelled a.s. (and mice) 5 ppm; 0.81 and 1.02 mg/kg bw 100 ppm; 16.8 and 21.5 mg/kg bw 2500 ppm; 434 and 475 mg/kg bw Single dose mice: 597 mg/kg bw Single dose rats: 9.4 mg/kg bw Unlabelled propiconazole and phenyl-^{14}C-propiconazole	DAR IIA 5.1.1/06 Acceptable
Oral (gavage) Not a guideline study non-GLP	Rat Sprague Dawley TifRAIf(SPF) males Triazole: 20 animals Phenyl: 3 animals	Single oral dose of 31.4 mg/kg bw Triazole-^{14}C-propiconazole in 1 ml vehicle and 32.5 mg/kg bw Phenyl-^{14}C-propiconazole in 1 ml vehicle.	DAR IIA 5.1.2/01 Acceptable
Oral (gavage) Not a guideline study (analysis of urine and feces collected in 5.1.2/01) non-GLP	Rat Sprague Dawley TifRAIf(SPF) males Triazole: 20 animals Phenyl: 3 animals	Single oral dose of 31.4 mg/kg bw Triazole-^{14}C-propiconazole 31.4 mg/kg bw in 1 ml vehicle and 32.5 mg/kg bw Phenyl-^{14}C-propiconazole 32.5 mg/kg bw in 1 ml vehicle.	DAR IIA 5.1.2/02 Acceptable
Oral non-GLP	Rat Tif:RAIf, Mouse Tif:MAGf males 6 mice and 6 rats/dose	0, 20, 80, 160 and 320 mg/kg propiconazole technical (purity 90,7%) Daily treatment (10 ml/kg) for 14 consecutive days	DAR IIA 5.8.6/02 Acceptable

Absorption

Oral

The study with orally administered triazole-^{14}C-propiconazole (IIA 5.1.1/01) in rat, at concentrations of 0.5 or 25 mg/kg bw indicated that, based on urinary excretion, over 50% of the triazole label was absorbed from the intestinal tract within 24 h. The orally administered concentrations of phenyl-^{14}C-propiconazole were 0.5 and 50 mg/kg bw in the first (IIA 5.1.1/02) and 0.5 mg/kg bw in the second experiment (IIA 5.1.1/03) which also studied biliary excretion. The intestinal absorption of the phenyl labelled active substance, based on urinary excretion, was again >50% within 24 h in study IIA 5.1.1/02. Study IIA 5.1.1/03 showed that absorption from the intestinal tract was in average 86% (75-91%) of the dose within 48 h. The mean biliary excretion accounted for 65% of the dose, whereas the average urinary excretion was only 20% within 48 h in this study.

After administration by a single intravenous dose (0.5 mg/kg bw) in rat of phenyl labelled active substance, radioactivity was recovered in urine (males 43% and females 46%) and faeces (males 42% and females 39%). The recoveries were about the same with intravenous and oral administration (IIA 5.1.1/02).

Absorption in mouse calculated on the basis of urinary and fecal excretion and on the amount remaining in tissue and carcass was > 70% in males and > 60% in females within 24 h (IIA 5.1.1/06).

Dermal

Dermal absorption was studied in two experiments with triazole-^{14}C-labelled propiconazole in rats. The rate of absorption through the skin was >60% at both administered doses (1 and 10 mg/kg bw) in the first experiment (IIA 5.1.1/04), with skin half life times around 24 h at the low dose (1.0 mg/kg bw) and 31 h at the high dose (10 mg/kg bw). More than 60% of the label was found in tissues and excreta at the low dose and about 50% at the high dose. In the second experiment (IIA 5.1.1/05) dermal absorption was found to be inversely related to the administered dose. After 24 h of exposure, 47, 10 and 1% of the applied dose (0.1, 1.0 or 10 mg/animal) was absorbed and distributed (found in blood, urine, feces and carcass), whereas at the same time, an increasing amount (10, 17 and 27%) was extractable from the skin. At all doses in the second experiment, approximately 50% of the radiolabel could be removed by washing after 24 h. The highest amounts of residues were found in kidney and liver of the dermally treated rats in the study IIA 5.1.1/04. Characterization of the metabolite pattern in this study indicated extensive metabolization, with no unchanged parent compound present in urine. The overall excretion pattern in urine was similar to the one observed with oral administration in rat, except that there were more aglyconic metabolites in dermally treated rats. No indication of cleavage between the phenyl and triazole rings was observed in metabolites from dermally treated rats.

Distribution

Residues in tissues were generally low in rats, with highest concentrations found in liver and kidney. The 50-fold difference between the high and low dose in study IIA 5.1.1/01 was reflected by a 40-fold difference in tissue residues, and the 100-fold difference in the orally administered dose in study IIA 5.1.1/02 was reflected by 52 - 86 and 42 - 98-fold differences in kidney and liver residues, respectively. The kinetic study (IIA 5.1.1/03) with 0.5 mg/kg bw phenyl labelled propiconazole in rat showed that residues at low administered doses of active substance were at their peak only one hour after treatment. Comparison of residues in tissues after i.v. or oral administration showed that the

radiolabel was found in more tissues after the i.v. dose, but the doses were similar to those observed at oral doses.

In mice, residual radioactivity remained low in the low dose groups, with slightly higher levels in females than in males (except in the kidneys) (IIA 5.1.1/06). The highest amounts of residues were generally found in kidneys and liver. Residues in male and female mice were similar to those found in male rats treated with a comparable dose of labelled propiconazole in the same study.

Metabolism

The preliminary characterization (IIA 5.1.2/01) of metabolic fractions excreted in urine and feces after oral administration of triazole-^{14}C- and phenyl-^{14}C-propiconazole in rats indicated the presence of some 20 different radiolabelled fractions in urine and at least 8 fractions in feces. About 5 of the urinary fractions appeared in concentrations higher than 5% of the total administered radiolabel, the major metabolite being fraction no. 8 which accounted for 24% of the total radioactivity. Some 80% of the urinary metabolites were acidic by nature, and about 12% of these were conjugated with sulfate and 9% with glucuronide. No unchanged parent compound was found in urine.

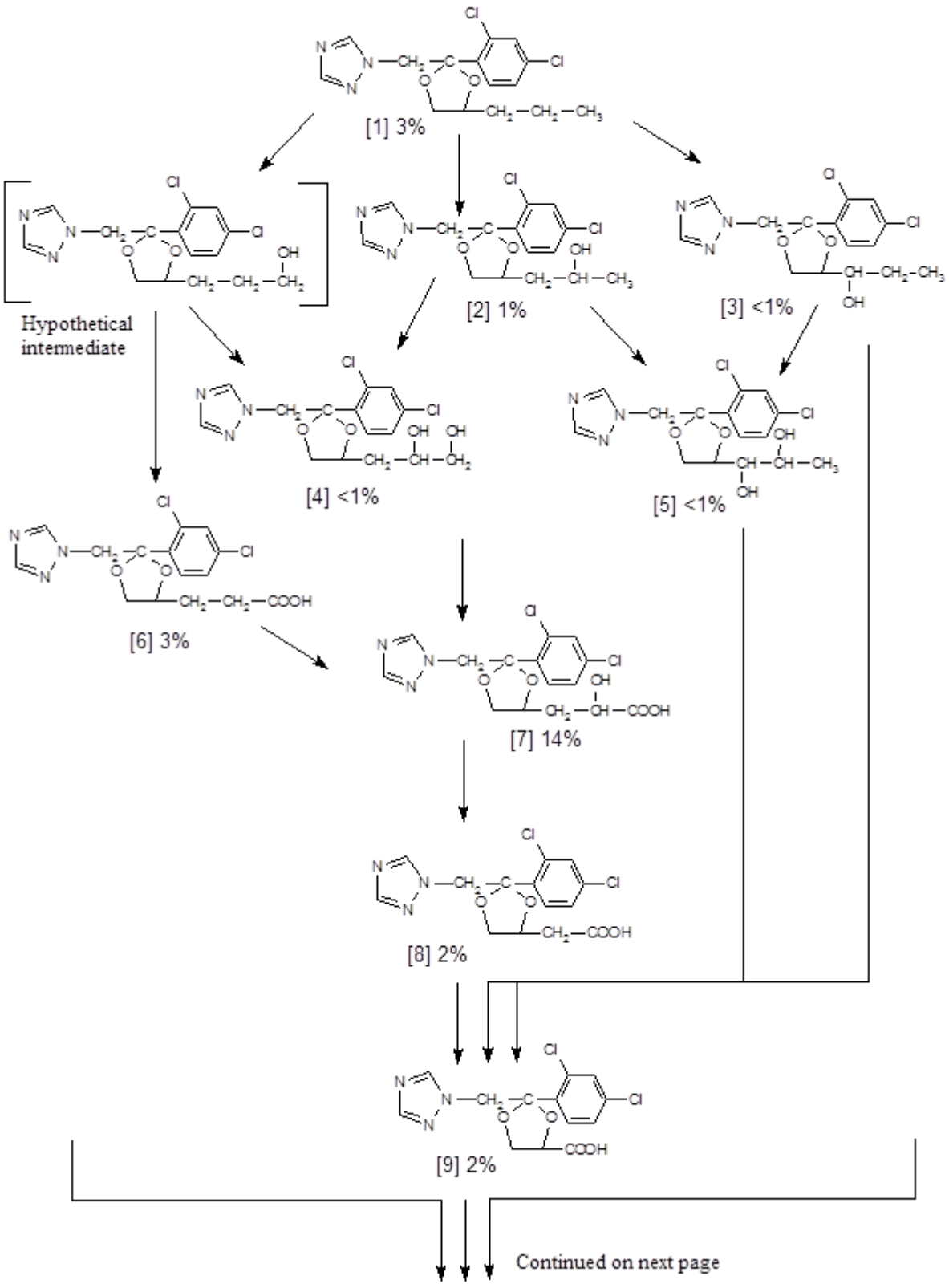
About 60% of the fecal metabolites were extractable with methanol/water. The 8 fractions found in the extract were less polar than the urinary metabolite fractions. Some 35% of the extractable fecal metabolites were acidic by nature. About 3% unchanged parent compound was found in feces.

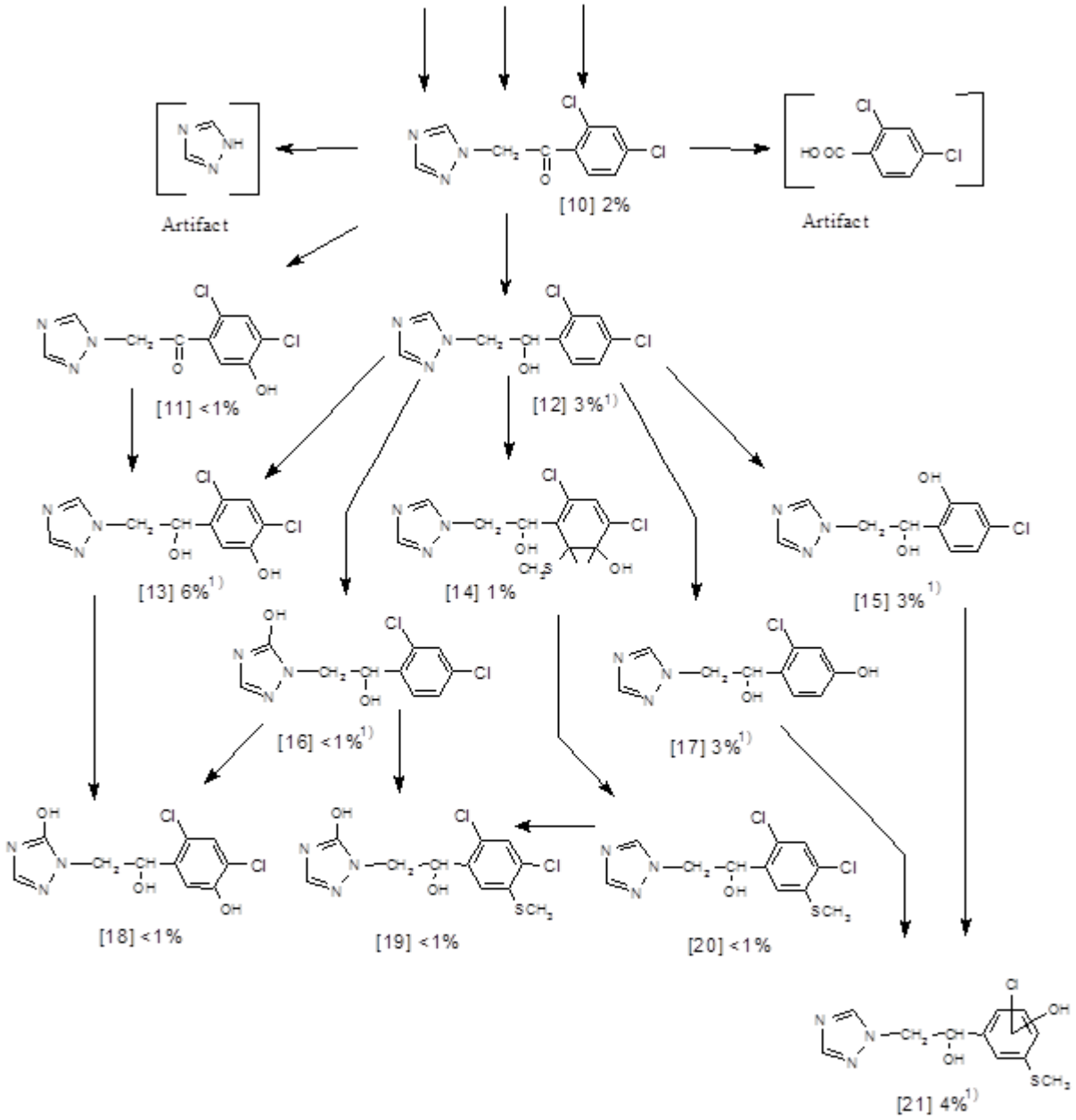
The preliminary study in rats indicated that the dioxolane ring was partly cleaved, but that most urinary and fecal metabolites have an intact bridge between the phenyl and triazole rings.

A closer characterization of the metabolites in urine and feces of rats treated with 31.4 µg/kg triazole-labelled propiconazole (IIA 5.1.2/01) using LC and HPLC separation tecniques and spectroscopy employing MS, EI-, FD-, HR-MS and NMR techniques in section IIA 5.1.2/02. The urine and feces collected one day after application contained about 45% and 36% of the triazole labelled dose, respectively. The proposed metabolic pathway of propiconazole in rat is presented in Figure 1. This metabolic pathway is a consensus presentation of the urinary and fecal excretion patterns, with the carboxylic compound [7] found in the acidic urinary fraction being the most abundant metabolite (14% of dose) in urine. The most frequent metabolites in feces were the neutral metabolites [3, 12, 13, 15, 17 and 18], comprising about 3% of the dose excreted in feces. About 5% of the parent compound was excreted unchanged in feces, and it is assumed that this amount moves directly from the stomach to the intestinal tract without being absorbed. No parent compound was present in urine, when the limit of detection was 0.1% with TLC.

Fractionation of urine from male and female mice treated with single doses of 5, 100 or 2500 ppm phenyl-^{14}C-labelled propiconazole showed, in all dose groups, 15-30 metabolite fractions. About 30% of the radiolabelled dose in the urine of male mice after 24 hours was accounted for by one metabolite which was identified as the glucuronic acid conjugate of 1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazole-1-yl)-ethanol. This metabolite accounted for only 15% of the radiolabelled dose in urine from female mice. In rats, only about 3% of the radiolabel was associated with this compound. The results indicate differences in the metabolic fate of propiconazole, both between sexes in mice and also between mouse and rat, with a more efficient cleavage of the dioxolan ring in mice, especially in males (IIA 5.1.1/06).

Figure 1: Proposed metabolism of propiconazole in rat. Values represent precent of dose.





Excretion

Urinary excretion in rats was higher than fecal excretion in study IIA 5.1.1/01, with 78% of the label eliminated within 24 h and 95% within 48 h (average total excretion). Study IIA 5.1.1/02 indicated equal extent of elimination through urine and feces both at oral administration and also at intravenous administration. The excretion rate was high also in study IIA 5.1.1/02, 70% was eliminated within 24 h and 95% within 48 h. In the third rat experiment (IIA 5.1.1/03), studying biliary excretion kinetics after administration of 0.5 mg labelled as per kg bw, 65% of the label was excreted with bile in cannulated male rats, 20% in urine and 6% in feces within 48 h.

Mice excreted most of the radiolabel (39-72%) in urine within 24 h (15-35% in feces), whereas rats in the same study eliminated about equal amounts of radioactivity in urine and feces (IIA 5.1.1/06).

4.1.2 Human information

No data are available.

4.1.3 Summary and discussion on toxicokinetics

Toxicokinetic studies in rat showed that most (>50%) of the orally administered propiconazole is rapidly absorbed from the intestinal tract (IIA 5.1.1/01, IIA 5.1.1/02). The rate of excretion is also high, around 95 % of the administered dose is found in urine and feces within 48 h (IIA 5.1.1/01, IIA 5.1.1/02). Propiconazole is efficiently metabolised; no unchanged parent compound is found in urine (IIA 5.1.2/01). The rate of skin absorption in rat, as a consensus of two independent studies, was fairly high, especially at relatively low doses (IIA 5.1.1/04). In one of the skin absorption studies, the rate of absorption was inversely related to the administered dose, showing an absorption rate close to 50 % at the lowest dose (0.1 mg/animal) (IIA 5.1.1/05).

Residue levels in tissues were fairly low in both rats and mice, with highest activities found in liver and kidneys (IIA 5.1.1/01, IIA 5.1.1/02, IIA 5.1.1/03, IIA 5.1.1/06).

Propiconazole was mostly excreted in urine, although the extent of fecal excretion rate was in many cases almost equal to urinary excretion. In rat propiconazole was excreted mainly as metabolites, with no parent compound found in urine. Feces contained about 3 % of unchanged propiconazole in rat (IIA 5.1.2/01). The metabolic reactions in rat included mainly oxidation and shortening of the n-propyl side chain, cleavage of the dioxolan ring and hydroxylation of the triazole and phenyl rings. Some evidence of cleavage of the alkyl bridge between the phenyl and triazole rings was also found. Metabolism study in mice indicated efficient cleavage of the dioxolan ring with subsequent conjugation reactions, especially in males (IIA 5.1.1/06). Significantly higher activities of UDP-glucuronosyltransferase and glutathione S-transferase were also measured in male mice than in male rats in the liver enzyme induction study (IIA 5.8.6/02).

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method **Guideline** **GLP**	**Species** **Strain** **Sex** **no/group**	**Doses** **Route** **Exposure period**	**Results/ Remarks**	**Reference**
OECD Guideline 425 (Acute Oral Toxicity: Up-and-Down Procedure) EPA OPPTS 870.1100 (Acute Oral Toxicity) GLP	Rat RjHan:WI Female 1-3/group	Test material: Propiconazole tech. 95.2% technical 175, 550, 2000 mg/kg bw oral: gavage Single dose	LD_{50}: 550 mg/kg bw (female) based on mortalities at 2000, 550 and 175 mg/kg (2/2, 1/3 and 0/1 respectively.)	dRAR B.6.2.1.3
Similar to OECD Guideline 401	Mouse Tif:MAG (SPF) 5 male and 5 female	Test material: Propiconazole. 93% pure 800, 1500, 2500 or 3000 mg/kg Oral: gavage Single dose	LD_{50}: 1490 mg/kg bw (male/female) based on: 1/10 deaths at 800 mg/kg, 4/10 deaths at 1500 mg/kg, 9/10 deaths at 2500 mg/kg and 10/10 deaths at 3000 mg/kg	DAR IIA 5.2.1/02
OECD Guideline 403 (Acute Inhalation Toxicity) GLP	Rat Tif:RAIf (SPF) 5 male and 5 female	Test material: Propiconazole. 91.1% pure 0, 5836 ±186 mg/m3 Inhalation: Aerosol (nose only) 4 hours	LC50 (4 h): > 5800 mg/m³ air (male/female) based on no mortality at the limit dose	Key study DAR IIA 5.2.3/01
OECD Guideline 402 (Acute Dermal Toxicity) EPA OPPTS 870.1200 (Acute Dermal Toxicity) EC 440/2008 GLP	Rat RjHan:WI Male/female 5 male and 5 female	Test material: Propiconazole tech. 95.2% pure 5000 mg/kg Coverage: semi-occlusive 24 hours	LD50: > 5000 mg/kg bw (male/female) based on no mortality at the limit dose	dRAR B.6.2.2.3
OECD Guideline 402 (Acute Dermal Toxicity)	Rat Tif:RAIf (SPF) Male/female 5 male and 5 female	Test material 93% pure 3000, 4000 mg/kg Coverage: occlusive 24 hours	LD50 : > 4000 mg/kg bw (male/female) based on no mortality at the limit dose	Key Study DAR II A 5.2.2 / 01

Method **Guideline** **GLP**	**Species** **Strain** **Sex** **no/group**	**Doses** **Route** **Exposure period**	**Results/ Remarks**	**Reference**
Protocol similar to OECD 402 (Acute Dermal Toxicity), but only 3 animals per sex	Rabbit New Zealand White 3 male and 3 female	Purity not reported 0, 2000, 6000 mg/kg bw Coverage: occlusive 24 hours	LD50: > 6000 mg/kg bw (male/female) based on no mortality at the limit dose	DAR II A 5.2.2/02

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In a rat acute oral study (dRAR B.6.2.1.3), a total of six, fasted, 8-11 week old, female RjHan:WI rats were given a single oral dose of propiconazole (95.2%) in 2% carboxymethyl cellulose at doses of 2000, 550 or 175 mg/kg bw. Single animals were dosed sequentially at no less than 24 hour intervals. Surviving animals were observed individually after dosing at 30 minutes, 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter. Body weight was measured on Day -1 and just before treatment and weekly after. All surviving animals were examined macroscopically at the end of the study. There were mortalities at 2000 mg/kg bw (2/2) and at 550 mg/kg bw (1/3). The animal treated at 175 mg/kg bw survived. Clinical signs including decreased activity, prone position, incoordination, lateral position and hunched back were observed in both animals treated at 2000 mg/kg bw. One animal dosed at 2000 mg/kg also showed decreased body temperature and noisy respiration. Clinical signs in animals treated at 550 mg/kg bw included decreased activity (3/3), incoordination (3/3), hunched back (2/3), piloerection (2/3), lateral position (2/3) and decreased body temperature (2/3). No clinical signs were observed in the animal dosed at 175 mg/kg. The acute oral median lethal dose (LD_{50}) of propiconazole tech. was calculated to be 550 mg/kg bw in female RjHan:WI rats.

In an acute oral toxicity study (DAR IIA 5.2.1/02), groups of five male and five female, young adult, Tif:MAG (SPF) mice received a single gavage dose of 800, 1500, 2500 or 3000 mg/kg of propiconazole (purity not reported), following an overnight fast. Animals were observed for 14 days. Body weights were measured on Day 1 (prior to dosing), 7 and 14. Clinical signs were recorded throughout the study. All animals were examined macroscopically following death, or at the end of the study. There were deaths at all dose levels: 0, 4, 4 and 5 males, and 1, 0, 5 and 5 females at 800, 1500, 2500 and 3000 mg/kg respectively. Clinical signs from immediately after dosing included sedation, dyspnoea, ruffled fur and lateral, ventral and/or curved body position. Surviving animals recovered within 10-11 days and gained body weight. No abnormal findings were observed at necropsy. The acute oral LD_{50} of propiconazole was 1490 (1138-1875) mg/kg bw in male and female mice.

4.2.1.2 Acute toxicity: inhalation

A group of five male and five female Tif:RAI f (SPF) rats was exposed nose-only for a single four-hour period to propiconazole aerosol at a concentration of 5836 ±186 mg/m^3 (DAR IIA 5.2.3/01). A control group of five rats per sex was exposed to the vehicle, absolute ethanol GR (as a 30% w/w solution). There were no deaths. Signs of systemic toxicity (ruffled fur, dyspnoea, abnormal body positions and reduced activity) were seen in were seen in control and, to a greater severity, in test animals. All animals had fully recovered by day 9 of the study. All animals gained body weight during the study. The males exposed to the test article experienced a significantly lower body weight gain compared to controls. There were no macroscopic abnormalities at the examination post mortem. The acute inhalation LC_{50} of propiconazole after a 4 hour nose-only exposure was greater than 5800 mg/m^3 in male and female rats.

4.2.1.3 Acute toxicity: dermal

In an acute dermal toxicity study (dRAR B.6.2.2.3), a group of five male and five female, young adult, RjHan:WI rats was exposed by a single semi-occlusive dermal application of 5000 mg/kg of propiconazole (95.2%) for 24 hours to approximately 10% of the total body surface. Body weight and clinical observations were recorded throughout the study. Animals were observed for 14 days. All animals were examined macroscopically at the end of the study. The acute dermal LD_{50} of propiconazole tech. was greater than 5000 mg/kg bw in male and female RjHan:(WI) Wistar rats.

In an acute dermal toxicity study (DARIIA 5.2.2 / 01), groups of five male and five female, young adult, Tif:RAIf (SPF) rats were exposed by a single occlusive dermal application of 3000 or 4000 mg/kg of propiconazole (purity 93%) for 24 hours to a clipped area on the back. Animals were observed for 14 days. Body weights were measured on Day 1 (prior to dosing), 7 and 14. Clinical signs were recorded throughout the study. All animals were examined macroscopically at the end of the study. There were no deaths. Clinical signs from 2 days after dosing included dyspnoea, ruffled fur and curved body position in both groups, with full recovery within 9 days. There was no effect on body weight. No abnormal findings were observed at necropsy. The acute dermal LD_{50} of propiconazole was greater than 4000 mg/kg bw in male and female rats.

In an acute dermal toxicity study (DAR IIA 5.2.2/02), groups of three male and three female, adult, New Zealand White rabbits were exposed by a single occlusive dermal application of 0, 2000 or 6000 mg/kg of propiconazole (purity not reported) for 24 hours to a clipped area on the back. Animals were observed for 14 days. There were no deaths. There were no clinical signs or effect on body weight. No abnormal findings were observed at necropsy. The acute dermal LD_{50} of propiconazole was greater than 6000 mg/kg bw in male and female New Zealand White rabbits.

4.2.1.4 Acute toxicity: other routes

No additional data are available

4.2.2 Human information

No data are available

4.2.3 Summary and discussion of acute toxicity

The acute oral toxicity of propiconazole was shown to be relatively low in both rats and mice. In the rat the lowest oral LD_{50} was 550 mg/kg bw and in the mouse 1490 mg/kg bw.

In a nose-only inhalation study, rats were exposed to a limit dose of 5836 mg/m^3 propiconazole aerosol for 4 hours. There were no deaths and clinical signs were similar to controls although body weight gain in the 14 days following exposure was reduced in treated animals. The LC_{50} was > 5800 mg/m^3.

The acute dermal toxicity was examined in 2 rat and 1 rabbit studies, which all demonstrated that propiconazole is of low toxicity. The LD_{50} was > 4000 mg/kg bw in all 3 studies.

4.2.4 Comparison with criteria

Via the oral route, the lowest LD_{50} was 550 mg/kg bw, therefore propiconazole should be classified acutely toxic via oral route. According to the CLP (Regulation (EC) No 1272/2008), propiconazole should be classified as Acute Tox. 4; H302, because the LD_{50} is within the limits 300 < ATE ≤ 2000 (oral, mg/kg bw). The minimum classification Acute Tox. 4* is thus considered confirmed.

Via the dermal route, the LD_{50} was >4000 mg/kg bw and therefore no classification is warranted under CLP.

Via the inhalation route the LC_{50} to rats was >5800 mg/m^3 and therefore no classification is warranted.

4.2.5 Conclusions on classification and labelling

CLP: Acute Tox 4; H302

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of propiconazole for acute oral toxicity as Category 4 on the basis of an acute oral toxicity up-and-down test (OECD TG 425) in rat showing an LD50 of 550 mg/kg bw.

The DS proposed no classification of propiconazole for acute dermal toxicity on the basis of two independent studies in rat showing LD50 higher than 4000 and 5000 mg/kg bw and a third study in rabbit showing an LD50 higher than 6000 mg/kg bw. In the three cases the studies were performed following OECD TG 402.

A single study of acute inhalation toxicity (OECD TG 403) showing an LC50 higher than 5800 mg/m3 made the DS conclude on no classification of propiconazole for acute toxicity by inhalation route.

Comments received during public consultation

Four Member State Competent Authorities (MSCAs) supported the classification proposed by the DS.

Assessment and comparison with the classification criteria

The three tables below summarise the acute oral, dermal and inhalation animal toxicity studies, respectively, that were assessed by the DS in the CLH report. No cases of poisoning of humans with propiconazole have been reported.

Table: Summary of acute oral toxicity studies with propiconazole. In all cases propiconazole was administered by gavage.

Method	Species Sex Nº group	Dose level	Results	Reference
OECD TG 425 (Up-and-Down Procedure) EPA OPPTS 870.1100 GLP	Rat RjHan:WI Females 1-3/group	175, 550, 2000 mg/kg bw	LD_{50} = 550 mg/kg bw Mortalities at 2000 (2/2) and 550 (1/3) mg/kg bw No mortality (0/1) at 175 mg/kg bw Clinical signs: decreased activity, prone position, incoordination, lateral position and hunched back were observed in both animals treated at 2000 mg/kg bw	dRAR B.6.2.1.3
Similar to OECD TG 401	Mouse Tif:MAG (SPF) 5 males and 5 females	800, 1500, 2500 or 3000 mg/kg	LD_{50} = 1490 mg/kg bw 1/10 deaths at 800 mg/kg, 4/10 deaths at 1500 mg/kg, 9/10 deaths at 2500 mg/kg, 10/10 deaths at 3000 mg/kg Clinical signs: sedation, dyspnoea, ruffled fur and lateral, ventral and/or curved body position	DAR IIA 5.2.1/02

Table: Summary of acute dermal toxicity studies with propiconazole.

Method	Species Sex Nº group	Dose level	Results	Reference
OECD TG 402 EPA OPPTS 870.1200 EC 440/2008 GLP	Rat RjHan:WI 5 males and 5 females	5000 mg/kg bw Coverage: semi-occlusive 24 hours	LD_{50} > 5000 mg/kg bw No mortalities	dRAR B.6.2.2.3
OECD TG 402	Rat Tif:RAIf (SPF) 5 males and 5 females	3000, 4000 mg/kg Coverage: occlusive 24 hours	LD_{50} > 4000 mg/kg bw No mortalities Clinical signs from 2 days after dosing included dyspnoea, ruffled fur and curved body position in both	DAR II A 5.2.2 / 01

			groups, with full recovery within 9 days.	
Similar to OECD TG 402	Rabbit New Zealand White 3 males and 3 females	0, 2000, 6000 mg/kg bw Coverage: occlusive 24 hours	LD_{50} > 6000 mg/kg bw No mortalities No clinical signs or effect on body weight. No abnormal findings were observed at necropsy.	DAR II A 5.2.2/02

Table: Summary of acute inhalation toxicity studies with propiconazole. The assay was performed with propiconazole of 91.1% purity.

Method	Species Sex Nº group	Dose level	Results	Reference
OECD TG 403 GLP	Rat Tif:RAIf (SPF) 5 males and 5 females	0, 5836 ± 186 mg/m3 Inhalation: Aerosol (nose only) 4 hours	LC50 (4 h): > 5800 mg/m³ No mortalities Signs of systemic toxicity (ruffled fur, dyspnoea, abnormal body positions and reduced activity) were seen in control and, with a greater severity, in test animals.	DAR IIA 5.2.3/01

Comparison with the criteria

The acute oral test in mouse yielded an LD_{50} of 1490 mg/kg bw. However, rat was noted to be the most sensitive species with a LD_{50} of 550 mg/kg bw. The classification should be based on the most appropriate sensitive species tested and in this case the LD_{50} for rat is within the limits 300 < LD_{50} ≤ 2000 mg/kg bw. Therefore, RAC is in agreement with the DS, and concludes on classification of propiconazole as **Acute Oral Toxicity Category 4 (H302: Harmful if swallowed).**

The limit concentration for triggering classification for the dermal route is 2000 mg/kg. The available information shows that doses of up to 4000-6000 mg/kg bw of propiconazole did not cause fatalities. Thus, RAC agrees with the DS that propiconazole **does not fulfil the criteria for classification for dermal acute toxicity.**

The limit concentration for triggering classification for the inhalation route is 5000 mg/m^3. The available information shows that a dose of 5800 mg/m^3 of propiconazole did not cause fatalities. Thus, RAC agrees with the DS that propiconazole **does not fulfil the criteria for classification for inhalation acute toxicity.**

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

This hazard was not reviewed in the CLH report.

Comments received during public consultation

One MSCA submitted a comment indicating that this hazard should be considered on the basis of a relevant FAO/WHO assessment [2]. The DS replied that this hazard had not been evaluated in the CLH dossier.

Assessment and comparison with the classification criteria

RAC can only form opinions on hazard classes that have been proposed for review in the CLH dossier and which proposal has been subject to public consultation. Therefore, RAC did not evaluate this hazard class.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

This hazard was not reviewed in the CLH report.

Comments received during public consultation

One MSCA submitted a comment indicating that this hazard should be considered on the basis of a relevant FAO/WHO assessment [3]. The DS replied that this hazard had not been evaluated in the CLH dossier.

Assessment and comparison with the classification criteria

[2] FAO Plant Production and Protection Paper, 178, 2004 - Pesticide residues in food – 2004 (Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO the Core Assessment Group)

[3] FAO Plant Production and Protection Paper, 178, 2004 - Pesticide residues in food – 2004 (Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO the Core Assessment Group)

RAC can only form opinions on hazard classes that have been proposed for review in the CLH dossier and which proposal has been subject to public consultation. Therefore, RAC did not evaluate this hazard class.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

4.6.1 Skin sensitisation

Following studies and references are taken from the Draft Assessment Report (DAR) and Competent Authority Report (CAR) of propiconazole.

Table 11: Summary table of skin sensitisation studies

Species	Method	Number of animals sensitised/total number of animals	Result	Reference
Guinea pig Himalayan Spotted (GOHI Ibm:GOHI (SPF)) 10 animals/ sex/ test group 5 animals/ sex/ vehicle control group	Guinea pig maximisation test OECD 406 Induction: intradermal 5% Propiconazole, and epicutaneous, vehicle peanut oil Challenge: epicutaneous 30% Propiconazole, occlusive, vehicle vaseline Test material: Propiconazole technical (purity 92,4%) GLP	Test group: 1st reading: 6/20, 24 h after challenge 2nd reading: 10/20, 48 h after challenge Vehicle control group: 1st reading: 0/10, 24 h after challenge 2nd reading: 0/10, 48 h after challenge	Sensitising	DAR IIA 5.2.6 Key study Acceptable
Guinea pig Pirbright White 10 animals/ sex/ group	Optimization test Similar to the method recommended in the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959), the US Association of Food and Drug Officials (AFDO). Induction: intradermal Challenge: intradermal and epicutaneous Propiconazole technical (purity 93 %) Vehicle: Propylene glycol, Vaseline non-GLP	Test group: After intradermal challenge: 2 / 20; 24 h after challenge; dose 0.1% After epidermal challenge: 3 / 19; 24 h after challenge; dose 10% Vehicle control group: After intradermal challenge: 4 / 19; 24 h after challenge. After epidermal challenge: 0 / 18; 24 h after challenge.	Not concluded	DAR IIA 5.2.6/01 Not acceptable

4.6.1.1 Non-human information

Guinea pig maximisation test (GPMT)

A skin sensitisation study in guinea pigs (DAR IIA 5.2.6) was performed in compliance with GLP and according to OECD 406 (Directive 96/54/EC B.6).

Pre-test

A pre-test to determine appropriate test substance concentrations for the main test was performed on 1 male and 1 female albino guinea pig (Himalayan Spotted [GOHI (SPF)]) per dose by giving one pair of injections (0.1 ml each) of 0.5, 1.0, 3.0 and 5.0% propiconazole technicalin peanut oil in the shaved neck-shoulder area of the test animals. One pair of FCA/physiological saline mixtures (1:1) and one pair of each test concentration were injected so that one of each pair was on each side of the spine. The test sites were examined 24 and 48 h after administration to determine the highest concentration to cause mild to moderate irritation at the test site. Epidermal induction concentrations were tested at concentrations of 30, 50 and 80% propiconazole in vaseline and 100% (undiluted) propiconazole in 1 male and 1 female guinea pig 7 days after the application of 2 pairs of intradermal injections of a 1:1 mixture of FCA/physiological saline. The test items were applied dermally with four Hill Top Chambers, 2 on each flank of both animals. The test sites were again examined 24 and 48 h after application for signs of irritation.

All intradermal induction doses (0.5, 1.0, 3.0 and 5.0%) of propiconazole technical caused irritation at the injection site. The 5% concentration was chosen for the main test because it caused only mild to moderate skin irritation and produced no clinical signs in the test animals. The highest tested dermal application dose (100%) caused mild to moderate irritation and was chosen for epidermal induction. The 30% concentration produced no irritation and was therefore chosen as the dermal challenge application dose. Positive skin reactions were noted in all animals of the test article group after epidermal induction. No skin reactions were observed among animals in the vehicle control group.

Main test

The main test was conducted with 10 vehicle control group animals and 20 animals in the test group receiving propiconazole technical. Three pairs of intradermal injections (0.1 ml/site) of 5% propiconazole technical in peanut oil were applied into the shaved areas in the back of the neck of the test animals so that one of each pair was on each side of the midline.

Vehicle group injections: 1. Adjuvant/ physiological saline mixture 1:1 (v/v) 2. Peanut oil 3. Peanut oil 50% (w/v) with 1:1 adjuvant/ physiological saline mixture.

Propiconazole technical injections: 1. Adjuvant/ physiological saline mixture 1:1 (v/v) 2. Propiconazole technical (5%) in peanut oil 3. Propiconazole technical (5%) in 1:1 adjuvant/ physiological saline mixture.

On day 8 after intradermal injection, a filter paper patch fully loaded with the test item (100% propiconazole technical), or vaseline vehicle alone (about 0.4 g), was applied to the skin of the test animals and held in place with occlusive dressing for 48 h. Application sites were examined 1h after removal of the dressing and rated for positive or negative skin irritation reactions.

On day 21, one chamber loaded with the test item (30% propiconazole technical in vaseline, about 0.35 ml) was placed on one shaved flank and one chamber loaded with vaseline alone was placed on

the other shaved flank of the test animals of both groups. The chambers were held in place with occlusive dressing for 24 h. Skin reactions were observed through 48 h after completion of the challenge application. Skin reactions were scored 24 and 48 h after removal of the dressing, and dermal reactions were graded according to the Draize scale.

Clinical signs were checked for daily, as were also mortalities. Body weights were measured and recorded immediately before treatment and at study termination.

Positive skin reactions in the main test following challenge application were observed in 2 males and 4 females of the test group animals 24 h after completion of the application, and in 5 males and 5 females at the 48 h examination. The rate of sensitization of propiconazole technical was therefore 50%. No reactions were observed in animals treated with vehicle control. There were no mortalities during the test, and no remarkable clinical observations were reported. Body weights of the test animals were not affected by treatment.

In conclusion, according to the acceptable GPMT propiconazole causes sensitization by skin contact in guinea pig.

Optimization test

Skin sensitisation was studied by using an optimization test (DAR IIA 5.2.6/01), which was considered to be acceptable according to an earlier version of OECD Guideline 406, but not according to the present OECD Guideline or according to directive 92/69/EEC B.6. Regardless of the employed method, the sensitivity of the strain of guinea pig employed in the skin sensitisation tests should be checked at regular intervals using a known sensitizer, and a satisfactory number of positive responses have to be obtained to assess the reliability of the test system. Information to that effect has not been provided.

GLP was not compulsory when the study was performed, and it was therefore done without written standard operating procedures or quality assurance inspections.

The test was performed on groups of 10 male and 10 female guinea pigs of the Pirbright white strain. The test material (propiconazole technical, purity 93%) was administered in diluted form in an amount of 0.1 ml. The pH value of the test material was around 5. During the induction period the animals received 10 intracutaneous injections of a 0.1% dilution of propiconazole in propylene glycol. During the second and third weeks of the induction period the test material was incorporated in a mixture of vehicle with complete Bacto adjuvant (complete Freund). After a two week long treatment free period one intracutaneous injection of the test dilution (0.1% propiconazole in propylene glycol) was given as first challenge. Ten days after the intracutaneous challenge injection a sub-irritant dose of the test compound (10% in vaseline) was applied epicutaneously under an occlusive dressing which was left in place for 24 hours.

Two vehicle control animals and one animal in the test group died during the study. The incidence of positive animals after the intradermal challenge injection was 4/19 in the vehicle control group. The test substance was considered to be allergenic by the intradermal route if, according to the exact Fisher's test for comparison of the basic probability of two binominal distributions, the number of positive animals in the test group differed significantly ($p<0.01$) from the number of "pseudo-positive" guinea pigs in the control group. In this study, 21% of the controls showed a non-specific positive reaction. The epicutaneous challenge concentration should be the highest amount of the test substance that produces no evidence of skin irritation in non-sensitized animals. Data from a small scale pilot study, where this concentration was determined, has not been provided.

The incidence of positive animals after intradermal challenge injection was 4/19 in the vehicle control group and 2/20 in the test group. The incidence of positive animals after epidermal challenge application was 0/18 in the vehicle control group and 3/19 in the test group.

In conclusion, the study is not considered acceptable, neither by the intradermal nor the topical route, because it contains methodological deviations (no positive controls), deaths of experimental animals, non-specific positive reactions in vehicle controls, and deficiencies in the determination of dose levels for topical challenge application.

4.6.1.2 Human information

Medical surveillance of employees in production, formulation and packaging plants covering the period of 1982 to 04/2000 revealed 4 cases (out of a total of 139 individuals) of local skin reactions. All four cases occurred during handling of propiconazole containing formulations (DAR IIA 5.9.1/01 and CAR IIIA 6.12.1/02). A few reported clinical cases do not give evidence of potential sensitising effects caused by propiconazole in humans. In a few cases, chest pain and local skin reactions has been experienced when exposure to the product containing propiconazole has occurred by inhalation or skin contact (DAR IIA 5.9). In an epicutaneous test with 1% technical grade propiconazole conducted in 1991 at the University of Göttingen in Germany with 20 human volunteers gave no evidence of sensitisation or skin irritation (DAR IIA 5.9.1/02).

Penagos *et al*. (2004) (CAR IIIA 6.12.2) studied the underlying causes for frequently occurring cases of irritant contact dermatosis and allergic contact dermatosis (ACD) among workers at a banana plant in Panama. Patch testing for a number of pesticides, including propiconazole (concentration of propiconazole was 0.44% w/w equivalent to 3.07 mg/ml), was used for differential diagnosis. 37/60 workers were diagnosed with pesticide dermatosis, 15 ACD positive cases were identified by patch testing. One of the ACD positive workers tested positive for propiconazole in the patch test.

Summary and discussion of skin sensitisation

Two *in vivo* studies, GPMT (IIA 5.2.6) and optimization test (IIA 5.2.6/01), have been conducted to study the skin sensitisation potential of propiconazole. The optimization test showed that propiconazole is a skin sensitiser but the study is not considered as acceptable. According to the acceptable GPMT propiconazole is a skin sensitiser. Available data reports only a few cases on humans where exposure to propiconazole has caused skin reactions. Overall, the human data does not give clear evidence of potential skin sensitising effects of propiconazole.

4.6.1.3 Comparison with criteria

Propiconazole has a harmonized classification as Skin Sens. 1; H317. Along with the new criteria in the 2nd ATP of CLP Regulation (286/2011) it is possible to classify sensitisers in sub-categories. Based on the GPMT a substance can be categorised into a sub-category 1B if the response is ≥ 30% at > 1% intradermal induction dose or into a sub-category 1A if ≥ 30% responding ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1% to < 1% intradermal induction dose. In the key GPMT study (IIA5.2.6) the response was 50% at 5% intradermal induction dose. However, there are no data available from studies that could show skin sensitising effects at lower doses and therefore the sub-category 1A cannot be excluded. In conclusion, based on the result of the GPMT study, propiconazole should be classified as Skin Sens. 1; H317 without sub-categorisation.

4.6.1.4 Conclusions on classification and labelling

Propiconazole is currently classified as Skin Sens. 1; H317. The available data does not warrant revision of the classification and therefore classification as **Skin Sens. 1; H317** should be retained.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed to retain the current classification in Annex VI of propiconazole as skin sensitizer Category 1 (H317) because the available data (an OECD TG 406 study showing 30% and 50% of sensitisation 24 hours and 48 hours after challenge with 30% propiconazole and a few reports of humans where exposure to propiconazole caused skin reactions) does not warrant revision of the assigned classification.

Comments received during public consultation

Three MSCAs supported the classification proposed by the DS.

A manufacturer commented agreeing with the proposal to retain Category 1. However, the company submitted a new study where an intradermal injection induction dose of 1% propiconazole caused 0% of sensitisation and therefore would allow discarding category 1A and instead specify the subcategory leading to Category 1B. The DS assessed the new study and noted that in the induction phase skin reactions observed in control animals were similar to the test animals, while 24 hours after challenge one control animal (that received only vehicle) showed significant dermal response. The DS considered the new study not acceptable for classification purposes because of the non-specific positive reactions in vehicle control animals. RAC also noted that no positive controls were included in this new study and therefore negative results might be interpreted as an intrinsic resistance of the animals to sensitisation.

Assessment and comparison with the classification criteria

The table below summarises the available animal studies with propiconazole.

Table: Summary of animal studies on skin sensitisation with propiconazole.

Method	Species Strain Nº/group	Dose levels	Results Conclusion	Reference Acceptability
Guinea pig maximisation test OECD TG 406 GLP	Guinea pig Himalayan Spotted (GOHI Ibm:GOHI (SPF)) 10 animals/sex/ test group 5 animals/sex/ vehicle control group	Day 0: Induction: intradermal 5% propiconazole, in peanut oil Day 8: Induction: 100% propiconazole (or vaseline) occlusive for 48 hours	Test group: 6/20 (24 h after challenge) 10/20 (48 h after challenge) Vehicle control: 0/10 (24 h after challenge)	DAR IIA 5.2.6 **Acceptable**

		Day 21: Challenge: 30% propiconazole (or vaseline) occlusive for 24 hours	0/10 (48 h after challenge) **SENSITISING**	
Optimization test Similar to the method recommended in the "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959), the US Association of Food and Drug Officials (AFDO). non-GLP	Guinea pig Pirbright White 10 animals/sex/ group	Day 0: Induction: intradermal injections 0.1% propiconazole in propylene glycol Day 10: Challenge: 10% propiconazole occlusive dressing in vaseline (24 hours)	Test group: 2/20 (after intradermal challenge) 3/19 (24 h after epidermal challenge) Vehicle control group: 4/19 (after intradermal challenge) 0/18 (48 h after challenge) **INCONCLUSIVE**	DAR IIA 5.2.6/01 **Not acceptable**

Comparison with the criteria

A preliminary test showed that an intradermal dose of 5.0% propiconazole caused mild to moderate irritation at the injection site with no clinical signs. The same preliminary study also showed that dermal application of 100% and 30% propiconazole caused mild to moderate irritation and no irritation, respectively. The main test was performed using intradermal induction with 5.0% propiconazole and a first epidermal occlusive challenge with 100% propiconazole 8 days after induction and a second epidermal occlusive challenge with 30% propiconazole. These conditions caused positive skin reactions in 2 males and 4 females of the test group animals 24 h after completion of the application, and in 5 males and 5 females at the 48 h examination. Therefore the rates of sensitization 24 and 48 hours after induction were 30 and 50%, respectively. No reactions were observed in animals treated with vehicle control. There were no mortalities during the test, and no remarkable clinical observations were reported. Body weights of the test animals were not affected by treatment.

A second skin sensitisation study by using an optimization test was available. This optimization test was considered to be acceptable according to an earlier version of OECD TG 406, but not according to the present OECD test guideline or according to directive 92/69/EEC B.6. Regardless of the employed method, two additional reasons for disregarding the results of this study were that the sensitivity of the strain of Guinea pig employed in the test has not been checked and that two vehicle control animals and one animal in the test group died during the study. In this optimization test the induction was performed with 10 intracutaneous injections of a 0.1% dilution of propiconazole in propylene glycol. During the second and third weeks of the induction period the test material was incorporated in a mixture of vehicle with complete Bacto adjuvant (complete Freund). After a two week long treatment free period one intracutaneous injection of the test dilution (0.1% propiconazole in propylene glycol) was given as first challenge. Ten days after the intracutaneous challenge injection a

sub-irritant dose of the test compound (10% in vaseline) was applied epicutaneously under an occlusive dressing which was left in place for 24 hours. The incidence of positive animals immediately after and 24 hours after the intradermal challenge injection was 16 and 16%, respectively; while an incidence of 21% in the vehicle control group was found immediately after challenge. Thus, this study was considered inconclusive and non-acceptable for classification purposes.

There is information on a few cases in humans where exposure to propiconazole has caused skin reactions: i) Medical surveillance of employees in production, formulation and packaging plants revealed 4 cases of local skin reactions among 139 exposed individuals during the period 1982-2000; ii) A few cases where chest pain and local skin reactions have been experienced when exposed via inhalation or skin contact to a product containing propiconazole have been reported; iii) One case of allergic contact dermatitis diagnosed through patch testing with 3.07 mg propiconazole/mL among 60 individuals. In contrast to the information suggesting certain capability of propiconazole to induce sensitisation in humans an epicutaneous test with 1% technical grade propiconazole conducted with 20 human volunteers gave no evidence of sensitisation or skin irritation.

Overall, the human data does not give clear evidence of a potential skin sensitising effect of propiconazole, while the valid Guinea pig maximisation test showed that an intradermal induction of 5% propiconazole sensitised 50% of animals 48 hours after challenge. This response is within the range required in the ECHA "Guidance on the Application of the CLP Criteria" for classifying propiconazole as Category 1B (because the response was higher than ≥ 30% with an intradermal induction dose higher than 1%). However, RAC notes that induction was not tested at concentrations of 1% and lower and therefore it is unknown if the response at 1% would have been higher than 60% (criteria requested for classification as Category 1A). Therefore, category 1A cannot be excluded with the available information and RAC supports the DS's opinion and concludes on classification of propiconazole a **Skin Sensitizer Category 1 (without sub-categorisation); H317 (May cause an allergic skin reaction).**

4.6.2 Respiratory sensitisation

No data available

4.7 Repeated dose toxicity

In addition to the repeated exposure studies, relevant findings of 4 carcinogenicity studies were taken into consideration. These studies are described in the carcinogenicity section (4.9) of this CLH report.

Table 12: Summary table of relevant repeated dose toxicity studies

Method **Species** **Strain No/sex/group**	**Doses** **Route** **Exposure period**	**NOAELs/NOAECs**	**Reference**
Oral			
Rat, Tif:RAIf (SPF) Repeated Dose 28-Day Oral Toxicity Study in Rodents Equivalent or similar to OECD 407 10 males and 10 females /group	0, 50, 150, 450 mg/kg/day Purity: 91.9% Oral: gavage Exposure: 28 days (daily)	NOAEL: 50 mg/kg bw/day for males based on histopathology and haematological effects at 150 mg/kg bw/day. NOAEL could not be set for females	DAR II A 5.3.1/01 Acceptable
Rat, Tif:RAIf (SPF) Repeated Dose 90-Day Oral Toxicity Study in Rodents Equivalent or similar to OECD 408 20 males and 20 females /group	0, 240, 1200 and 6000 ppm (nominal in diet) (0/0, 15.9/16.8, 76.1/77.6, 461.7.480.9 mg/kg/day males/females) Purity: 90% Oral: continuously in diet Exposure: 3 months	NOAEL: 240 ppm (males 15.9, females 16.8 mg/kg bw/day) based on slightly increased splenic haemosiderosis at 6000 ppm and decreased body weight at 1200 ppm in females.	DAR II A 5.3.2/01 Acceptable
2 year chronic toxicity/ carcinogenicity study in rats	This study is described in the carcinogenicity section of this CLH report		DAR II A 5.5/02 Acceptable
Mouse, Crl:CD-1®(ICR) BR (Swiss) Repeated Dose 90-Day Oral Toxicity Study in Rodents Equivalent or similar to EPA OPP 82-1/ OECD 408 GLP 20 males and 20 females /group	0, 20, 500, 850 (males only), 1450 (males only), 2500 ppm (0/0, 2.7/3.1, 65/85, 112/-, 194/-, 352/434 mg/kg/day males/females) Purity: 92% Oral: continuously in diet Exposure: 17 weeks	NOAEL males: 20 ppm (2.7 mg/kg bw/day) based on liver toxicity (liver weight, histopathological alterations) at 500 ppm NOAEL females: 500 ppm (85 mg/kg bw/day) based on liver toxicity (liver weight, histopathological alterations) at 2500 ppm	DAR II A 5.3.2/03 Acceptable Key study
Mouse, Crl:CD-1®(ICR) BR (Swiss) Repeated Dose 90-Day Oral Toxicity Study in Rodents Equivalent or similar to EPA OPP 82-1/ OECD 408 GLP 40 males /group	0, 20, 500, 850, 1450 and 2500 ppm (0, 2.8, 71, 121, 199 and 360 mg/kg/day) Purity: 92% Oral: continuously in diet Exposure: 13 weeks	NOAEL: 20 ppm (2.8 mg/kg bw/day) based on liver toxicity (liver weight, histopathological findings) at 500 ppm	DAR IIA 5.3.2/04 Acceptable Key study
2 year carcinogenicity study in mice	This study is described in the carcinogenicity section of this CLH report		DAR II A 5.5/03 and 5.5/04 Acceptable
18 Month Study in mice	This study is described in the carcinogenicity section of this CLH report		DAR II A 5.5/05, Acceptable

Method **Species** **Strain No/sex/group**	**Doses** **Route** **Exposure period**	**NOAELs/NOAECs**	**Reference**
In vivo (mouse) study on biochemical and cellular interactions in the liver	This study is described in the carcinogenicity section of this CLH report		DAR II A 5.8.6/01
Dog Beagle 92-days study in dogs, Equivalent or similar to OECD 409 4 males and 4 females /group	0, 50, 250 and 1250 ppm (0/0, 1.34/1.65, 6.89/7.56 and 35.28/35.74 mg/kg/day males/females) Purity: 88% Oral: continuously in diet Exposure: 92 days	NOAEL: >1250 ppm (no treatment related effects at any dose)	DAR IIA 5.3.2/02 Not acceptable
Dog Beagle 53 week study in dogs, Equivalent or similar to OECD 452 5 males and 5 females /group	0, 5, 50, 250 ppm (0/0; 0.17/0.19; 1.86/1.86; 8.43/8.86 mg/kg bw/day males/females) Purity: 90.2% Oral: continuously in diet Exposure: 53 weeks	NOAEL: >250 ppm (no treatment related effects at any dose)	DAR IIA 5.5/01 Not acceptable
Inhalation			
Rat, RAIf (SPF) Subchronic Inhalation Toxicity: 90-Day Equivalent or similar to OECD 413 20 males and 20 females /group	0 (air), 10 mg/m^3 acetone (vehicle control), 21±2, 85±7 or 191±10 mg/m^3 (mean achieved concentration) Purity: 91.9% Inhalation: aerosol (nose/head only) Exposure: 13 weeks (6 hours per day, 5 days per week)	NOEC males: 21 mg/m³ air based on: lower body weights at 85 mg/m3. NOEC females: < 21 mg/m³ based on lower body weights at 21 mg/m³	DAR II A 5.3.3/02 Acceptable
Dermal			
Rabbit, New Zealand White Repeated Dose Dermal Toxicity: 21-Day Study Equivalent or similar to OECD 410 10 males and 10 females /group	0, 200, 1000 and 5000 mg/kg/day (nominal per unit body weight) Purity: 91.9% Dermal: shaved skin of the back covered with a gauze and occlusive dressing intact and abraded skin. Exposure: 21 days,daily (6 hours, 5 times per week for 3 consecutive weeks, total of 15 treatments).	NOAEL (systemic): 200 mg/kg bw/day based on adverse clinical signs at 1000 mg/kg bw/day NOAEL (local): < 200 mg/kg bw/day (skin irritation)	DAR IIA 5.3.3/01 Acceptable
Rat (Hanlbm:WIST (SPF) male/female Equivalent or similar to OECD 410 GLP 10 males and 10 females /group	0, 10, 100, 1000 mg/kg/day (nominal per unit body weight) Purity: 92.4% Dermal: shaved skin skin of the back covered with an occlusive dressing Exposure: 4 weeks. Weeks 1-3: once daily, 5 days per week, week 4: once daily, 7 days per week i/e. total of 22 treatments.	NOAEL (systemic): 1000 mg/kg bw/day (no treatment related effects at any dose) NOAEL (local): 100 mg/kg bw/day based on minimal acanthosis at 1000 mg/kg bw/day.	dRAR B.6.3.3.1.2 Acceptable

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

28-day rat gavage study (DAR II A 5.3.1/01)

Table 13: Summary of main findings

<table>
<tr><th>Study</th><th>Main findings</th></tr>
<tr><td>Rat, Tif:RAIf (SPF)
Repeated Dose 28-Day Oral Toxicity Study in Rodents
Equivalent or similar to OECD 407
10 males and 10 females /group
0, 50, 150, 450 mg/kg/day
Purity: 91.9%
Oral: gavage
Exposure: 28 days (daily)</td><td>
<u>Organ weights:</u>
<table>
<tr><th></th><th></th><th>0 mg/kg/d</th><th>50 mg/kg/d</th><th>150 mg/kg/d</th><th>450 mg/kg/d</th></tr>
<tr><td rowspan="2">Absolute liver weight (g) (mean)</td><td>♂</td><td>13.812</td><td>13.468</td><td>18.239*</td><td>18.933*</td></tr>
<tr><td>♀</td><td>8.593</td><td>11.587*</td><td>13.582*</td><td>14.236*</td></tr>
<tr><td rowspan="2">Liver weight relative to body weight ratio (mean)</td><td>♂</td><td>3.983</td><td>3.943</td><td>5.161*</td><td>5.883*</td></tr>
<tr><td>♀</td><td>4.016</td><td>5.000*</td><td>5.788*</td><td>6.855*</td></tr>
<tr><td rowspan="2">Liver weight relative to brain weight ratio (mean)</td><td>♂</td><td>664.966</td><td>675.468</td><td>904.080*</td><td>974.031*</td></tr>
<tr><td>♀</td><td>480.132</td><td>620.104*</td><td>731.620*</td><td>762.519*</td></tr>
</table>
* = significant difference compared to the control group (p<0.05)
<u>Histopathology:</u>
50 mg/kg bw/day:
No changes observed
150 mg/kg bw/day:
Minimal hypertrophy of hepatocytes (8/10 females and 4/10 males). Small focus organising necrosis in liver parenchyma (1/10 males).
450 mg/kg bw/day:
Minimal/moderate hypertrophy of hepatocytes (10/10 males and 10/10 females).
Multiple recent areas of necrosis in liver parenchyma (3/10 females).
</td></tr>
</table>

Animals were examined twice per day for mortality, once daily for clinical signs and body weight and food consumption were measured weekly. The eyes were examined and hearing checked prior to and at the end of the treatment period. At the end of the treatment period animals were fasted overnight. All surviving animals were bled under ether anaesthesia and subjected to detailed autopsy. Liver, adrenals, brain, heart, kidneys and gonads were weighed and a comprehensive range of tissues taken and preserved. Three samples of liver from each rat were examined histopathologically. Histopathological examinations were not performed to other organs.

Food consumption was reduced in 450 mg/kg bw/day females. Females at this dose level showed also sedation, dyspnea and ruffled fur during the first week of treatment. Liver weights (absolute and relative to body weight and brain weight) were statistically significantly increased in 150 and 450 mg/kg bw/day males and in all treated female groups in a dose-related manner. There was minimal or moderate hypertrophy of the hepatocytes in all males and females dosed 450 mg/kg bw /day and minimal hypertrophy in 4/10 male and 8/10 female rats at 150 mg/kg bw/day. In 3/10 females at 450 mg/kg bw/day, multiple, recent areas of necrosis in the liver parenchyma were observed. In one male at 150 mg/kg bw/day one small focus of necrosis was seen. The histopathological appearances of the liver in 50 mg/kg bw/day rats were comparable to controls. The NOAEL for propiconazole was 50 mg/kg bw/day for males. Due to the effects observed in liver in all dose levels the NOAEL-value could not be determined for female rats in this study. In the 450 mg/kg bw/day group females, the erythrocyte counts, hemoglobin and hematocrit values were reduced. In this dose group the number of thrombocytes showed an increasing trend in males. Chloride and glucose concentrations were

increased in females at 450 mg/kg bw/day. Both females and males (450 mg/kg bw/day) showed increases in the alfa2-globulin fraction, but females also showed decreasing gamma-globulin fractions. These haematological and blood chemistry changes were not considered to indicate toxicological concern, but were regarded as signs of slight anaemia.

90-day rat feeding study (DAR II A 5.3.2/01)

Table 14: Summary of main findings

Study	Main findings
Rat, Tif:RAIf (SPF) Repeated Dose 90-Day Oral Toxicity Study in Rodents Equivalent or similar to OECD 408 20 males and 20 females /group 0, 240, 1200 and 6000 ppm (nominal in diet) (0/0, 15.9/16.8, 76.1/77.6, 461.7.480.9 mg/kg/day males/females) Purity: 90% Oral: continuous in diet Exposure: 3 months	**240 ppm (males 15.9 mg/kg bw/day, females 16.8 mg/kg bw/day):** No effects **1200 ppm (males 76.1 mg/kg bw/day, females 77.6 mg/kg bw/day):** Body weight: ↓ both sexes (males 2.4%, 4.7% and 2.5% at weeks 2, 4 and 12; females 1.8%, 4.9% and 7.4% at weeks 2, 4 and 12, respectively). **6000 ppm (males 461.7 mg/kg bw/day, females 480.9 mg/kg bw/day):** Body weight: ↓ both sexes (males 13.0%, 16.8% and 21.7% at weeks 2, 4 and 12; females 8.2%, 10.6%, 18.7% at weeks 2, 4 and 12, respectively). Histopathology: slightly ↑ haemosiderosis in spleen of 20/20 females.

Mortality, clinical observations, body weights and food consumption were measured throughout the study. Blood samples were collected from all animals at 4, 8 and 13 weeks for haematology and clinical chemistry analysis; urinalysis was done during the weeks of blood collection. Brain, heart, liver, kidneys, adrenals and gonads were weighed. A comprehensive range of organs and tissues from all animals was examined histopathologically.

There was no mortality due to the administration of propiconazole. The body weight gains of all high dose (6000 ppm, 461.7/480.9 mg/kg bw/day; males/females) males and females and intermediate group (1200 ppm, 76.1/77.6 mg/kg bw/day; males/females) females were significantly reduced. Due to the reduced body weight gain the mean food conversion of the high dose (6000 ppm) males and females was slightly increased. The results of the haematological investigation, blood chemistry data and the urinalysis were generally unremarkable for both treated rats and controls. The terminal body weights of all high dose males and females and the intermediate dose females were significantly reduced. Organ weights and organ to body weight alteraltions were observed, however these differences were considered secondary to reductions in body weight. Slight or minimal focal hemosiderosis of the spleen was observed in all the females of the control group. The severity (moderate or marked) of this finding was increased in females of the high dose group. No other treatment-related gross or microscopic changes were found. The NOAEL was 240 ppm of propiconazole corresponding to 15.9 mg/kg bw/day for males and 16.8 mg/kg bw/day for females.

90-day mouse feeding study (DAR IIA 5.3.2/03)

Table 15: Summary of main findings

Study	**Main findings**

Mouse, Crl:CD-1®(ICR) BR (Swiss)

Repeated Dose 90-Day Oral Toxicity Study in Rodents Equivalent or similar to EPA OPP 82-1/ OECD 408 GLP

20 males and 20 females /group

0, 20, 500, 850 (males only), 1450 (males only), 2500 ppm (0/0, 2.7/3.1, 65/85, 112/-, 194/-, 352/434 mg/kg/day males/females)

Purity: 92%

Oral: continuously in diet

Exposure: 17 weeks

		20 animals per dose					
		0 ppm	**20 ppm**	**500 ppm**	**850 ppm**	**1450 ppm**	**2500 ppm**
Absolute and relative liver weights	♂			↑	↑	↑	↑
	♀				-	-	↑
Enlarged liver	♂	1	0	0	0	14 **	20 **
	♀	0	0	0	-	-	8 *
Focal discoloration of the liver	♂	0	0	0/	2	5	6 *
	♀	0	0	0	-	-	3
Hepatocellular hypertrophy	♂	0	0	4	14**	20 **	20 **
	♀	0	0	0	-	-	17 **
Hepatocellular necrosis	♂	1	0	2	4	8*	12 **
	♀	0	0	0	-	-	6 *
Hepatocellular necrosis of individual cells	♂	0	0	0	0	2	12 **
	♀	0	0	0	-	-	1
Hepatocellular necrosis of multi and/or individual cells	♂	1	0	2	4	10 **	18 **
	♀	0	0	0	-	-	6 *
Hepatocellular vacuolation	♂	0	0	6 *	2	3	10 **
	♀	0	0	0	-	-	2
Hepatocellular vacuolation of individual cells	♂	0	0	0	0	0	6*
	♀	0	0	0	-	-	1
Hepatocellular vacuolation multi and/or individual cells	♂	0	0	6 *	2	3	16 **
	♀	0	0	0	-	-	3
Serum cholesterol	♂				↓	↓	↓
	♀				-	-	
Alanine aminotransferase (ALT)	♂					↑	↑
	♀				-	-	↑
Aspartate aminotransferase (AST)	♂						
	♀				-	-	↑

* = significant difference compared to the control group ($p<0.05$)
** = significant difference compared to the control group ($p<0.01$)
↑/↓ = statistically significant increase/decrease

Mortality, clinical observations, body weights and food consumption were measured throughout the study. Blood samples were collected from all animals prior to necropsy for clinical chemistry

analysis. Livers were weighed and examined histopathologically. Brain was weighted. Other organs were not weighed or examined histopathologically.

There was no mortality due to the administration of propiconazole. A statistically significant negative trend of the mean body weights was observed in male mice. No consistent effect on body weights were noted in female mice. There were no treatment related effects on food consumption or feed efficiency. Increased liver weights (absolute and relative to body or brain) were noted for males at 500 ppm (65 mg/kg bw/day) and for females at 2500 ppm (434 mg/kg bw/day). Further evidence of hepatocellular toxicity was manifested as decreased serum cholesterol at ≥ 850 ppm and increased alanine aminotransferase at 1450 ppm (194 mg/kg bw/day) in males but not at 2500 ppm (352 mg/kg bw/day). Increased AST and ALT were observed at 2500 ppm in females. Histopathological examination of the livers revealed biologically and statistically significantly increased incidences of hepatocellular hypertrophy in males at ≥ 850 ppm and in females at 2500 ppm; necrosis in males at ≥ 1450 ppm and in females at 2500 ppm; and, hepatocellular vacuolation in males at 2500 ppm (352 mg/kg bw/day). The severity of hepatocellular necrosis was observed to be slight or very slight. In conclusion, propiconazole caused liver toxicity in both sexes of CD-1 mice after 17 weeks of treatment. The NOAEL was 20 ppm in male mice (2.7 mg/kg bw/day) and 500 ppm in female mice (85 mg/kg bw/day).

90-day mouse feeding study (DAR II A 5.3.2/04)

Table 16: Summary of main findings

Study	**Main findings**						
Mouse, Crl:CD-1®(ICR) BR (Swiss) Repeated Dose 90-Day Oral Toxicity Study in Rodents Equivalent or similar to EPA OPP 82-1/ OECD 408 GLP 40 males /dose 0, 20, 500, 850, 1450 and 2500 ppm (0, 2.8, 71, 121, 199 and 360 mg/kg/day) Purity: 92% Oral: continuously in diet Exposure: 13 weeks	* = significant difference compared to the control group (p<0.05)						
		40 animals per dose					
		0 ppm	**20 ppm**	**500 ppm**	**850 ppm**	**1450 ppm**	**2500 ppm**
	Absolute and/or relative liver weights			↑	↑	↑	↑
	Enlarged liver	0	0	0	14 **	32 **	40 **
	Discoloration of the liver	0	0	0	1	2	5
	Discoloration of the liver, pale	0	0	0	0	0	2
	Focal discoloration	0	0	0	6 *	8 **	8 **
	Friability	0	0	2	1	3	5
	Prominent lobular architecture	0	0	0	0	6 *	18 **
	Hepatocellular hypertrophy	0	0	10 **	35 **	40 **	40 **
	Hepatocellular necrosis	1	0	4	8 *	16 **	17 **
	Hepatocellular necrosis of individual cells	0	0	0	1	23 **	29 **
	Hepatocellular necrosis of multi and/or individual cells	1	0	4	9 *	31 **	34 **
	Hepatocellular vacuolation	0	1	2	5	11 **	22 **
	Hepatocellular vacuolation of individual cells	0	0	0	0	4	11 **
	Hepatocellular vacuolation multi and/or individual cells	0	1	2	5	15 **	33 **
	Hepatocellular mineralization	0	0	0	3	2	9 **
	Serum cholesterol			↓	↓	↓	↓
	ALT					↑	↑
	Sorbitol dehydrogenase (SDH)				↑	↑	↑
	** = significant difference compared to the control group (p<0.01) ↑/↓ = statistically significant increase/decrease						

Ten animals per group were killed after 4 and 8 weeks on study and 20 animals after 13 weeks of treatment. Mortality, clinical observations, body weights and food consumption were measured throughout the study. Blood samples were collected from all animals prior to necropsy for clinical chemistry analysis. Livers and brains were weighed. Other organs were not weighed or examined histopathologically. Livers from all animals were examined histopathologically. Since no time-related differences of treatment-induced microscopic observations were observed, the data from all animals from each dose group were summarised together in the original study report.

There was no mortality due to the administration of propiconazole. Significant decreases in mean body weights were observed in the 2500 ppm (360 mg/kg bw/day) treated males during the first 8 weeks of the study. A statistically significant negative trend of the mean body weights was observed. There were no treatment-related effects on food consumption or feed efficiency. Increased liver weight (absolute and relative to body or brain) was noted at ≥ 500 ppm after 13 weeks of treatment. Absolute liver weights and liver to brain weight ratios were increased at ≥ 850 ppm after 4 or 8 weeks of treatment while liver to body weight ratios were increased at ≥ 500 ppm. Further evidence of hepatocellular toxicity was manifested as decreased serum cholesterol at ≥ 500 ppm, increased ALT at ≥ 1450 ppm and increased SDH at ≥ 850 ppm. Histopathological examination of livers revealed statistically significantly increased incidences of hepatocellular hypertrophy in males at ≥ 500 ppm; necrosis at ≥ 850 ppm; hepatocellular vacuolation at ≥ 1450 ppm and mineralization at 2500 ppm. There was a dose-related trend for increased severity of the hypertrophy, necrosis, individual cell necrosis and vacuolation. The severity of hepatocellular necrosis observed was mostly slight or very slight and only in 2 rats the necrosis was scored as moderate. No severe necrosis was observed. The NOAEL was 20 ppm (2.8 mg/kg bw/day).

92-days study and 53 week study in dog (DAR IIA 5.3.2/02; DAR IIA 5.5/01)

Two subchronic toxicity studies of propiconazole in dogs have been conducted. In the 3-month study propiconazole (purity 88%) was administered to groups of 6 male and 6 female Beagle dogs in diet at dose levels of 0, 50, 250 and 1250 ppm (1.34/1.65, 6.89/7.56 and 35.28/35.74 mg/kg/day males/females) for up to three months. According to study report, no treatment-related gross or histopathological changes were observed. At the highest dose slight stomach effects (granular surfaces in the pyloric and prepyloric parts of the stomach) were observed, however these were likely due to local irritation caused by propiconazole. In the one year study propiconazole (purity 90.2%) was administered to groups of 5 male and 5 female Beagle dogs in diet at dose levels of 0, 5, 50 and 250 ppm (0/0, 0.17/0.19, 1.86/1.86, 8.43/8.86 mg/kg bw/day males/females). No treatment-related effects were reported in this study. Since neither of the studies reported any clear dose-related effects, it is concluded that the selected dose-ranges were too low. Thus these studies are not considered acceptable.

4.7.1.2 Repeated dose toxicity: inhalation

13-week rat inhalation study (DAR II A 5.3.3/02)

Table 17: Summary of main findings

Study	Main findings
Rat, RAIf (SPF) Subchronic Inhalation Toxicity: 90-Day Equivalent or similar to OECD 413 20 males and 20 females /group 0 (air), 10 mg/m^3 acetone (vehicle control), 21±2, 85±7 or 191±10 mg/m^3 (mean achieved concentration) Purity: 91.9% Inhalation: aerosol (nose/head only)	**21 mg/m^3:** Body weight: ↓ by 5.9%, 7.3% and 5.3% females at weeks 4, 8 and 12 respectively. **85 mg/m^3:** Body weight: ↓ by 2.2%, 6.3%, 6.5% and 3.5% in males at weeks 1, 4, 8 and 12 respectively; 4.5% and 4.1% in females at weeks 4 and 8 respectively. **191 mg/m^3:** Body weight: ↓ by 4.8%, 5.3% , 3.5% and 0.5% in males at weeks 1, 4, 8 and 12 respectively; 1.1%, 5.9%, 7.3% and 5.3% in females at weeks 1, 4, 8 and 12 respectively.

Study	Main findings
Exposure: 13 weeks (6 hours per day, 5 days per week)	

Mortality, clinical observations, body weights and food consumption were measured throughout the study. Blood samples were collected from all animals at 6 and 13 weeks for haematology and clinical chemistry analysis. Brain, heart, liver, lungs and kidneys were weighed. A comprehensive range of organs and tissues from all animals was examined histopathologically.

No treatment-related mortality or clinical symptoms were observed throughout the study. There was a trend towards reduced body weight gain which was clearer for females than males. The development of the reduced body weight gain was, however, not dose-related and is therefore of limited toxicological importance. In 3 males of the highest dose group minimal to moderate focal fatty change of the liver was observed. There were no other relevant histopathological or gross pathological findings. A NOEC of propiconazole for male rats was established at 21 mg/m^3. No NOEC was determined for female rats, since body weight reduction was noted even at the lowest exposure level.

4.7.1.3 Repeated dose toxicity: dermal

21-day rabbit dermal study (DAR IIA 5.3.3/01)

Table 18: Summary of main findings

Study	Main findings
Rabbit, New Zealand White Repeated Dose Dermal Toxicity: 21-Day Study Equivalent or similar to OECD 410 10 males and 10 females /group 0, 200, 1000 and 5000 mg/kg/day (nominal per unit body weight) Purity: 91.9% Dermal: Shaved skin of the back covered with a gauze and occlusive dressing intact and abraded skin). Exposure: 21 days,daily (6 hours, 5 times per week for 3 consecutive weeks, total of 15 treatments.)	**200 mg/kg bw/day**: Irritation: slight skin irritation, no differences between intact and abraded skin (mean erythema score (days 2-20) 1.6 intact, 1.8 abraded. Mean oedema score (days 2-20) 1.1 intact, 1.1 abraded). **1000 mg/kg bw/day**: Irritation: slight skin irritation, no differences between intact and abraded skin (mean erythema score (days 2-20) 2.1 intact, 2.0 abraded. Mean oedema score (days 2-20) 1.5 intact, 1.5 abraded). Clinical observations: from day 4, sedation (10/10 males, 10/10 females). Days 15-21, ruffled fur, tremor, dyspnoea and diarrhoea (number of animals affected not reported). Histopathology: moderate acanthosis and hyperkeratosis of epidermis, chronic inflammatory infiltration in dermis (4/10 male, 4/10 females). Minimal or slight focal acanthosis of epidermis, slight chronic infiltration in dermis (5/10 males). **5000 mg/kg bw/day**: Irritation: slight skin irritation, no differences between intact and abraded skin (mean erythema score (days 2-20) 2.1 intact, 1.9 abraded. Mean oedema score (days 2-20) 1.6 intact, 1.6 abraded). Clinical observations: from day 4, ruffled fur (10/10 males, 10/10 females), dyspnoea (10/10 males, 10/10 females), tremor (10/10 males, 10/10 females), ataxia (10/10 males, 10/10 females) and sedation (10/10 males, 10/10 females). Body weight: ↓ by 8.6%, 8.7% and 12.5% days 12, 15 and 19 respectively in females.

Study	Main findings
	Histopathology: marked acanthosis and hyperkeratosis of epidermis, chronic inflammatory infiltration and focal fibrosis in dermis (6/10 males, 9/10 females). Necrosis of epithelium, marked acanthosis and hyperkeratosis of epidermis, focal fibrosis and chronic infiltration in dermis (3/10 males, 1/10 females). Minimal or slight focal acanthosis of epidermis, slight chronic infiltration in dermis (1/10 males).

Groups of 10 male and 10 female New Zealand White rabbits were given 6 hour dermal applications of 0, 200, 1000 or 5000 mg/kg bw/day of propiconazole, five days per week for three consecutive weeks (a total of 15 applications) (II 5.3.3/01). Half the animals were abraded (penetrating the stratum corneum) immediately before the start of treatment and then weekly throughout the study. Clinical observations, skin irritation, body weight, food consumption, haematology and clinical chemistry parameters were assessed. All animals were examined post mortem and histopathological examination of selected tissues and organs was performed.

There was no mortality during the study. Adverse clinical signs (ruffled fur, dyspnea, tremor, ataxia and sedation) were seen in all animals at 5000 mg/kg bw/day and sedation was seen in all animals at 1000 mg/kg bw/day. A decrease in erytrocyte and hemoglobin values was detected in high dose females. The toxicological significance of this finding is, however, not clear. In the males at the same dose an increased plasma bilirubin concentration was seen (control mean/max: 1.1/1.6 µmol/l, 5000 mg/kg bw/day mean/max: 4.5/17.2 µmol/l). Also γ-GT was increased in both sexes, but no other enzymes were affected.

At necropsy, increased absolute (not in females) and relative liver weights were noted at 5000 mg/kg bw/day. Liver to body weight ratios were 128 % and 125 % of the control values in males and females, respectively. There were no clear histopathological effects in the liver. At the site of dermal application, local changes showing focal acanthosis and hyperkeratosis of the epidermis and chronic inflammatory infiltration in the dermis were observed at histopathological examination. The intensity of these dermal changes was dose related. In 4 out of 20 rabbits from the 5000 mg/kg bw/day group, focal necrosis and ulceration of the epidermis were observed. The intensity of dermal changes in the majority of rabbits (19/20) from the lowest (200 mg/kg bw/day) dose group was only slight and similar to those seen in several (6/20) control rabbits and attributed to the local irritation due to repeated abrasion and application of an occlusive bandage. It was concluded that the 21 day dermal NOAEL of propiconazole for male and female rabbits was below 200 mg/kg bw/day for local effects and 200 mg/kg bw/day for systemic effects.

28-day rat dermal study (dRAR B.6.3.3.1.2)

Table 19: Summary of main findings

Study	Main findings
Rat (Hanlbm:WIST (SPF) male/female Equivalent or similar to OECD 410 GLP 10 males and 10 females /group 0, 10, 100, 1000 mg/kg/day (nominal per unit body weight) Purity: 92.4%	**10 mg/kg bw/day**: No adverse effects observed. **100 mg/kg bw/day**: No adverse effects observed. **1000 mg/kg bw/day**: Blood clinical chemistry: protein ↑ 5.6% (F), globulin ↑ 8.2% (F), A/G ratio ↓ 4.5% (F), cholesterol ↑ 28.6% (F), chloride ↓ 2.3% (F). Organ weights: absolute liver weight ↑ 18.9% (M), 14.0% (F), liver weight relative to bw ↑15.1% (M), 9.5% (F).

Study	Main findings
Exposure: 4 weeks (once daily, 5 times per week for 3 consecutive weeks, 7 days/week for week 4) i/e. total of 22 treatments.	Histopathology: acanthosis (minimal) at application site (8/10 F compared to 1/10 F controls).

Clinical signs, body weight, food consumption, food efficiency and mortality were monitored throughout for all animals. Detailed clinical observations were performed weekly. Haematological and blood chemistry analyses were performed at the end of the study. At sacrifice, animals were examined macroscopically and organ weights were recorded. Comprehensive range of organs and tissues were collected and prepared for histopathological examination. Organs and tissues were examined microscopically from all control and high dose animals and skin at the application site was examined from all animals. The test item was distributed homogeneously in the dosing preparations and was stable at the targeted concentrations.

There were no mortalities and no clinical signs or signs of local irritation. There were no treatment-related effects on body weight development, food consumption, food utilisation, ophthalmoscopy or haematological parameters. Slightly higher values for protein and globulin with an associated decrease in the albumin to globulin ratio were recorded for females at 1000 mg/kg bw/day. In addition, females at 1000 mg/kg bw/day had an increased cholesterol level and females at 100 and 1000 mg/kg bw/day had lower values for chloride. In the absence of a dose-response relationship, significantly lower glucose value in females at 100 mg/kg bw/day and significantly higher globulin value in females at 10 mg/kg bw/day were considered not relevant. In both sexes the mean liver weight was increased at 1000 mg/kg bw/day. At the application site, increased incidence of minimal acanthosis was seen in females at 1000 mg/kg bw/day. The NOAEL in this 28-day dermal rat study was 100 mg/kg bw/day.

4.7.1.4 Repeated dose toxicity: other routes

No data on other routes for repeated dose toxicity available.

4.7.2 Human information

No data available.

4.7.3 Other relevant information

No data available

4.7.4 Summary and discussion of repeated dose toxicity

The sub-chronic toxicity of propiconazole was investigated in rat (oral, dermal and inhalation routes), rabbit (dermal), mouse (oral) and dog (oral). The dog studies were not considered acceptable due to poorly selected dose ranges.

Liver was identified as the main target organ of propiconazole toxicity. In the 17-week study male mice were more susceptible to propiconazole than female mice (II 5.3.2/03). Significant liver weight increases were noted in males at 500, 850, 1450 and 2500 ppm (65, 112, 194 and 352 mg/kg/day respectively). In males serum cholesterol was significantly decreased at ≥ 850 ppm and ALT levels were increased at 1450 ppm. Incidence of hepatocellular hypertrophy was increased at ≥ 850 ppm; necrosis at ≥ 1450 ppm and, hepatocellular vacuolation at 2500 ppm in males. In females receiving

2500 ppm (434 mg/kg bw/day) liver weight, ALT and AST levels and incidences of hepatocellular hypertrophy and necrosis were significantly increased. In both sexes, the severity of necrosis was scored to be slight or very slight.

A 13-week study was conducted on male mice (II 5.3.2/04). Increased liver weight (absolute and relative to body or brain) was noted at ≥ 500 ppm. Further evidence of hepatocellular toxicity was manifested as decreased serum cholesterol at ≥ 500 ppm, increased ALT at ≥ 1450 ppm and increased SDH at ≥ 850 ppm. Hepatocellular hypertrophy was observed at ≥ 500 ppm; necrosis at ≥ 850 ppm; hepatocellular vacuolation at ≥ 1450 ppm and mineralization at 2500 ppm. The severity of hepatocellular necrosis was mostly slight or very slight and only in 2 rats the necrosis was scored as moderate (at the dose levels 1450 and 2500 ppm). Some treatment-related findings of hepatotoxicity were observed in the 18-months carcinogenicity study in male mice (IIA, 5.5/05). Reduced plasma cholesterol levels, increased liver weights andincidence of hepatocellular hypertrophy were reported in 500 and 850 ppm (59.0 and 107.6 mg/kg/day) dose groups. Slightly increased incidence of hepatocellular necrosis was observed at 850 ppm after 9 weeks treatment. Yet after 52 weeks or 18 months similar findings were not reported. In a two-year carcinogenicity study in mice hepatocellular necrosis was observed at the highest dose group (2500 ppm) after one year (incidence: 4/9) (II 5.5/02). However, in terminal sacrifice there was no difference between treated and control groups. In amechanistic carcinogenicity study, where mice were dosed up to 60 days, minimal hepatocellular necrosis was reported in several mice at the dose level 850 ppm and almost all mice had minimal to moderate hepatocellular necrosis at the dose level 2500 ppm. In conclusion, the severity of hepatocellular necrosis observed was only very slight to moderate and the necrosis appeared to subside by the time. Thus, although these findings were observed below the cut off value for STOT RE 2 classification (100 mg/kg bw/day, oral, rat), they are not considered to indicate toxicologically significant functional disturbance or morphological change in the liver.

In rat, the main treatment-related effects in oral repeated dose toxicity studies were increased liver weight, hepatic hypertrophy and increased incidence of foci of enlarged liver cells. No other signs of hepatotoxicity were observed.

In a 90 day inhalation study, rats were exposed nose only to propiconazole aerosol. The only adverse effect reported was decrease in body weights. Two dermal studies are available, one in rabbits and one in rats. In rabbits there were dose related topical changes at 1000 and 5000 mg/kg bw/day. In the rat study, systemic effects at 1000 and 100 mg/kg bw/day were limited to changes in a few blood chemistry parameters in females only and increased liver weights in both sexes at 1000 mg/kg bw/day.

4.7.5 Comparison with the criteria of repeated dose toxicity findings relevant for classification as STOT RE

According to the CLP regulation classification in STOT RE is required for substances that cause: "… consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health."

Liver was clearly identified as the main target organ of toxicity of propiconazole in rats and mice. In addition to the increases in the liver weight, also histopathological alterations such as hepatocellular hypertrophy, vacuolation, and single cell necrosis were observed. These findings were supported by the evidence of functional impairments such as altered clinical chemistry and marker enzymes. Single cell necrosis in the liver was observed below the cut-off values set for the STOT RE 2 classification (oral, rat; $10 < C \leq 100$ mg/kg bw/d). However, necrosis was scored to be very slight to moderate in

severity and it was primarily observed in short term studies. Therefore these findingsare not considered to indicate toxicologically significant functional disturbance or morphological change in the liver. Therefore it is considered appropriate that no classification is proposed.

4.7.6 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification

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

Summary of the Dossier Submitter's proposal

The DS assessed sub-chronic toxicity studies of propiconazole in rat (oral, dermal and inhalation routes), rabbit (dermal route) and mouse (oral route). The DS concluded that liver is the main target organ of propiconazole, inducing weight increases, hepatocellular hypertrophy and, to a minor extension, necrosis and vacuolation. This hepatocellular toxicity was also accompanied with alterations in clinical chemistry. However, the DS did not consider the findings of toxicological significance and did not propose propiconazole to be classified for STOT RE.

Comments received during public consultation

One MSCA supported the 'no classification' proposed by the DS.

Assessment and comparison with the classification criteria

The three tables below summarises the main relevant findings in the repeated toxicity studies with propiconazole after oral, inhalation and dermal exposure, respectively.

Table: Summary of the repeated toxicity studies by oral route with propiconazole.

Method	Species Strain Nº/group Dose levels	Results	Reference
Repeated dose 28-day oral toxicity study in rodents Equivalent or similar to OECD TG 407 Gavage	Rat Tif:RAIf (SPF) 10 males and 10 females /group 0, 50, 150, 450 mg/kg bw/d	<u>50 mg/kg bw/d:</u> No histopathological changes Females: Absolute liver weight, liver weight/body weight ratio and liver weight/brain weight ratio 135%, 125% and 129% of control, respectively <u>150 mg/kg bw/d:</u> Minimal hypertrophy of hepatocytes (8/10 females and 4/10 males) Small focus organising necrosis in liver parenchyma (1/10 males)	DAR II A 5.3.1/01

		Females: Absolute liver weight, liver weight/body weight ratio and liver weight/brain weight ratio 158%, 144% and 152% of control, respectively Males: Absolute liver weight, liver weight/body weight ratio and liver weight/brain weight ratio 132%, 130% and 136% of control, respectively <u>450 mg/kg bw/d:</u> Minimal/moderate hypertrophy of hepatocytes (10/10 males and 10/10 females) Multiple recent areas of necrosis in liver parenchyma (3/10 females) Females: Absolute liver weight, liver weight/body weight ratio and liver weight/brain weight ratio 166%, 171% and 159% of control, respectively Males: Absolute liver weight, liver weight/body weight ratio and liver weight/brain weight ratio 137%, 148% and 146% of control, respectively **NOAEL males = 50 mg/kg bw/d** **LOAEL females = 50 mg/kg bw/d**	
Repeated dose 90-day oral toxicity study in rodents Equivalent or similar to OECD TG 408 Oral (diet)	Rat Tif:RAIf (SPF) 20 males and 20 females /group 0, 240, 1200 and 6000 ppm (nominal in diet) Males: 0, 15.9, 76.1, 461.7 mg/kg bw/d Females: 0, 16.8, 77.6, 480.9 mg/kg bw/d	<u>240 ppm:</u> No effects <u>1200 ppm:</u> Body weight: ↓ both sexes (males 2.4%, 4.7% and 2.5% at weeks 2, 4 and 12; females 1.8%, 4.9% and 7.4% at weeks 2, 4 and 12, respectively) <u>6000 ppm:</u> Body weight: ↓ both sexes (males 13.0%, 16.8% and 21.7% at weeks 2, 4 and 12; females 8.2%, 10.6%, 18.7% at weeks 2, 4 and 12, respectively) Histopathology: slightly ↑ haemosiderosis in spleen of 20/20 females **NOAEL = 240 ppm (males 15.9 and females 16.8 mg/kg bw/d)**	DAR II A 5.3.2/01
2-year chronic toxicity/ carcinogenicity study OECD TG 453 (1981)	Rat Sprague Dawley CD	<u>100 ppm:</u> No effects <u>500 ppm:</u> Reduced body weight gain and food utilization in females and lower adrenal weights in males	DAR IIA 5.5/02

<table>
<tr>
<td>GLP

Oral (diet)</td>
<td>80 males and 80 females

0, 100, 500, 2500 ppm.

Males: 0, 3.60, 18.10, 96.46 mg/kg bw/d

Females: 0, 4.57, 23.32, 130.63 mg/kg bw/d</td>
<td>Transient alterations in clinical chemistry

<u>2500 ppm:</u>
Reduced body weight gain and food consumption in both sexes

Increased liver weights in both sexes (16% males, 19% females) and increased incidence of foci of enlarged liver cells in females (13/71 vs 1/70 in control)

Lower kidney weight and adrenal weight (with no histopathological associated changes)

Transient alterations in clinical chemistry.

NOAEL = 100 ppm (males 3.60 and females 4.57 mg/kg bw/d)</td>
<td></td>
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<tr>
<td>Repeated dose 90-day oral toxicity study in rodents

Equivalent or similar to EPA OPP 82-1/ OECD TG 408

GLP

Oral (diet)</td>
<td>Mouse

Crl:CD-1®(ICR) BR (Swiss)

20 males and 20 females /group

0, 20, 500, 850 (males only), 1450 (males only), 2500 ppm

Males: 0, 2.7, 65, 112, 194, 352 mg/kg bw/d

Females: 0, 3.1, 85, 434 mg/kg bw/d</td>
<td><u>Hepatic effects in males:</u>

<table>
<tr><td></td><td colspan="4">Dose (ppm)</td></tr>
<tr><td></td><td>500</td><td>850</td><td>1450</td><td>2500</td></tr>
<tr><td>Absolute and relative weight</td><td>↑</td><td>↑</td><td>↑</td><td>↑</td></tr>
<tr><td>Enlarged</td><td>0</td><td>0</td><td>14</td><td>20</td></tr>
<tr><td>Focal discoloration</td><td>0</td><td>2</td><td>5</td><td>6</td></tr>
<tr><td>Hypertrophy</td><td>4</td><td>14</td><td>20</td><td>20</td></tr>
<tr><td>Necrosis</td><td>2</td><td>4</td><td>8</td><td>12</td></tr>
<tr><td>Necrosis of individual cells</td><td>0</td><td>0</td><td>2</td><td>12</td></tr>
<tr><td>Necrosis of multi and/or individual cells</td><td>2</td><td>4</td><td>10</td><td>18</td></tr>
<tr><td>Vacuolation</td><td>6</td><td>2</td><td>3</td><td>10</td></tr>
<tr><td>Vacuolation of individual cells</td><td>0</td><td>0</td><td>0</td><td>6</td></tr>
<tr><td>Vacuolation multi and/or individual cells</td><td>6</td><td>2</td><td>2</td><td>16</td></tr>
</table>

<u>Hepatic effects in females:</u>

<table>
<tr><td></td><td colspan="2">Dose</td></tr>
<tr><td></td><td>500 ppm</td><td>2500 ppm</td></tr>
<tr><td>Absolute and relative weight</td><td></td><td>↑</td></tr>
<tr><td>Enlarged</td><td>0</td><td>8</td></tr>
<tr><td>Focal discoloration</td><td>0</td><td>3</td></tr>
<tr><td>Hypertrophy</td><td>0</td><td>17</td></tr>
<tr><td>Necrosis</td><td>0</td><td>6</td></tr>
<tr><td>Necrosis of individual cells</td><td>0</td><td>1</td></tr>
</table>
</td>
<td>DAR II A 5.3.2/03</td>
</tr>
</table>

<table>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><td>Necrosis of multi and/or individual cells</td><td>0</td><td>6</td></tr>
<tr><td>Vacuolation</td><td>0</td><td>2</td></tr>
<tr><td>Vacuolation of individual cells</td><td>0</td><td>1</td></tr>
<tr><td>Vacuolation of multi and/or individual cells</td><td>0</td><td>3</td></tr>
</table>
Inconsistent clinical changes in males

Increased ALT and AST at 2500 ppm in females (severity not reported)

NOAEL males = 20 ppm (2.7 mg/kg bw/d)

NOAEL females = 500 ppm (85 mg/kg bw/d)
</td>
<td></td>
</tr>
<tr>
<td>Repeated dose 90-day oral toxicity study in rodents

Equivalent or similar to EPA OPP 82-1/ OECD TG 408

GLP

Oral (diet)</td>
<td>Mouse

Crl:CD-1®(ICR) BR (Swiss)

40 males /group

0, 20, 500, 850, 1450 and 2500 ppm

0, 2.8, 71, 121, 199 and 360 mg/kg bw/d</td>
<td>
Clinical chemistry alterations at 500 ppm and onwards (severity not reported)
<table>
<tr><td></td><td colspan="4">Dose (ppm)</td></tr>
<tr><td></td><td>500</td><td>850</td><td>1450</td><td>2500</td></tr>
<tr><td>Absolute and relative weight</td><td>↑</td><td>↑</td><td>↑</td><td>↑</td></tr>
<tr><td>Enlarged</td><td>0</td><td>14</td><td>21</td><td>40</td></tr>
<tr><td>Focal discoloration</td><td>0</td><td>6</td><td>8</td><td>8</td></tr>
<tr><td>Prominent lobular architecture</td><td>0</td><td>0</td><td>6</td><td>18</td></tr>
<tr><td>Hypertrophy</td><td>10</td><td>35</td><td>40</td><td>40</td></tr>
<tr><td>Necrosis</td><td>4</td><td>8</td><td>16</td><td>17</td></tr>
<tr><td>Necrosis of individual cells</td><td>0</td><td>1</td><td>23</td><td>29</td></tr>
<tr><td>Necrosis of multi and/or individual cells</td><td>4</td><td>9</td><td>31</td><td>34</td></tr>
<tr><td>Vacuolation</td><td>2</td><td>5</td><td>11</td><td>22</td></tr>
<tr><td>Vacuolation of individual cells</td><td>0</td><td>0</td><td>4</td><td>11</td></tr>
<tr><td>Vacuolation multi and/or individual cells</td><td>2</td><td>5</td><td>15</td><td>33</td></tr>
<tr><td>Mineralization</td><td>0</td><td>3</td><td>2</td><td>9</td></tr>
</table>
NOAEL = 20 ppm (2.8 mg/kg bw/d)
</td>
<td>DAR IIA 5.3.2/04</td>
</tr>
<tr>
<td>2-year carcinogenicity study
OECD TG 451

GLP

Oral (diet)</td>
<td>Mouse

CD-1

64 males and 64 females</td>
<td><u>500 ppm:</u>
Increased (123% of controls) interim absolute and relative liver weights (males)

Incidences of hepatocyte enlargement in 39/62 males

<u>2500 ppm:</u></td>
<td>DAR IIA 5.5/03

DAR IIA 5.5/04</td>
</tr>
</table>

	0, 100, 500 and 2500 ppm Males: 0, 10, 49, 344 mg/kg bw/d Females: 0, 11, 56, 340 mg/kg bw/d	Reduced body weights (11-16%) and weight gain (20-38%) in both sexes Increased terminal absolute (227% of controls) and relative (263% of control) liver weights (males) Increased terminal absolute (151% of controls) and relative (175% of control) liver weights (males) Incidences of hepatocyte enlargement (54/64 males and 43/64 females) Hepatocyte vacuolation (39/64 females) Inflammatory cell and chronic infiltration in 44/64 males Pigmented Kupffer cells in 44/64 males ALT, AST and ALP increases and cholesterol reductions **NOAEL (non-carcinogenic) = 100 ppm (males 10.04 mg/kg bw/d, males and females 10.79 mg/kg bw/d)**	
18-month study in CD-1 male mice OECD TG 451 GLP Oral (diet)	Mice CD-l (ICR) BR 80 males/group 0, 100, 500 and 850 ppm 0, 11, 59, 108 mg/kg bw/d	<u>500 ppm:</u> Reductions of cumulative mean body weight gain between weeks 13-50 (6.9-8.8%), but no significant changes in mean body weight or body weight gains beyond the first year Liver weight/body weight was increased by 13% at week 53 Hepatocellular hypertrophy in 6/10 animals by week 9 and in 28/50 animals at the end of the study Transient decreases in cholesterol levels <u>850 ppm:</u> Reductions of cumulative mean body weight gain between weeks 13-50 (5-15%), but no significant changes in mean body weight or body weight gains beyond the first year Liver weight/body weight was increased by 33% at week 9, by 29% at week 53 and by 20% at the end of the study Absolute liver weight was increased by 32% at week 9, by 11% at week 53 and by 19% at the end of the study Hepatocellular hypertrophy in 10/10 animals by week 9, in 8/10 by week 53 and in 29/50 animals at the end of the study	DAR IIA 5.5/05

		Kupffer cell pigmentation in 11/50 animals at the end of the study Transient decreases in cholesterol and increases in sorbitol dehydrogenase activity levels **NOAEL (non-carcinogenic) = 100 ppm (11.0 mg/kg bw/d)**	
92-day study in dogs Equivalent or similar to OECD TG 409 Oral (diet)	Dog Beagle 4 males and 4 females /group 0, 50, 250 and 1250 ppm Males: 0, 1.34, 6.89 and 35.28 mg/kg bw/d Females: 0, 1.65, 7.56 and 35.74 mg/kg bw/d	No treatment related effects at any dose **NOAEL > 1250 ppm (35.28 mg/kg bw/d in males and 35.74 mg/kg bw/d in females)**	DAR IIA 5.3.2/02
53-week study in dogs Equivalent or similar to OECD TG 452 Oral (diet)	Dog Beagle 5 males and 5 females /group 0, 5, 50, 250 ppm Males: 0, 0.17, 1.86, 8.43 mg/kg bw/d Females: 0, 0.19, 1.86, 8.86 mg/kg bw/d	No treatment related effects at any dose **NOAEL > 250 ppm (8.43 mg/kg bw/d in males and 8.86 mg/kg bw/d in females)**	DAR IIA 5.5/01
Two-generation reproduction study Draft OECD TG 418 Equivalent or similar to OECD TG 416 GLP	Rat Charles River CD strain 15 males and 30 females	<u>500 ppm:</u> Liver hypertrophy in males F_0 (13/15) and F_1 (5/15) Liver hypertrophy in females F_1 (15/30) Liver vacuolation in F_1 (8/15) males <u>2500 ppm:</u> ↓23% and ↓19% of total bodyweight gain in F_0 and F_1 parental females at the end of the study	DAR IIA 5.6.1/01

Oral (diet)	0, 100, 500 and 2500 ppm Males: 0, 8.4, 48.8 and 214.9 mg/kg bw/d Females: 0, 9.7, 43.7 and 242.9 mg/kg bw/d	Severe incidence of liver hypertrophy in males F_0 (14/15), F_1 (15/15), F_{1b} (10/10), F_{2b} (10/10) Severe incidence of liver hypertrophy in females F_0 (29/30), F_1 (29/30), F_{1b} (8/10), F_{2b} (9/10) Severe incidence of liver vacuolation in F_0 (14/15) and F_1 (11/15) males and in F_1 (10/30) females **NOAEL parental toxicity: 100 ppm (8.4 mg/kg bw/d males and 9.7 mg/kg bw/d females)**	
Developmental toxicity OECD TG 414 GLP Gavage Daily treatment on days 6-15 of gestation	Rat Crl:COBS CD (SD) BR VAF/PLUS) 30 females 0, 30, 90, 360/300 mg/kg bw/d The high dose was reduced to 300 mg/kg bw/d due to severe signs of maternal toxicity	<u>90 mg/kg bw/d:</u> Transient reduction in maternal food consumption and body weight gain but no significant differences by day 20 <u>360 mg/kg bw/d:</u> During the first week of treatment: lethargy, ataxia and salivation, and signs of rales, prostration, hypothermia and bradypnea. The toxic signs decreased immediately following the lowering of the dose level to 300 mg/kg bw/d on the sixth day of dosing Transient reduction in maternal food consumption and body weight gain but no significant differences by day 20 **NOAEL maternal toxicity: 90 mg/kg bw/d**	DAR IIA 5.6.2/01
Teratology study Modified OECD TG 414 GLP Gavage Daily treatment on days 6-15 of gestation	Rat Crl:COBS CD (SD) BR VAF/PLUS 0, 300 mg/kg bw/d	<u>300 mg/kg bw/d:</u> ↓17% corrected maternal body weight gain in the period 0-20 days Clinical signs: ataxia, comatose, lethargy, prostration, salivation, altered respiration 2 deaths Transient (days 6-16) decrease in maternal food consumption but similar to control in periods 0-6 and 16-20 days) **LOAEL maternal toxicity: 300 mg/kg bw/d**	DAR IIA 5.6.2/02 Acceptable
Teratology study OECD TG 414/EPA OPP 83-3 GLP Gavage	Rabbit New Zealand White 19 females	<u>100 mg/kg bw/d:</u> One death for unknown cause <u>250 mg/kg bw/d:</u> Maternal food consumption reduced between 24-37% in the period between 7-21 days of gestation but not in the period between days 5-6 and 20-29	DAR IIA 5.6.2/03

Daily treatment on days 7-19 of gestation	0, 100, 250, 400 mg/kg bw/d	<u>400 mg/kg bw/d:</u> Maternal food consumption reduced between 34-57% in the period between 7-21 days of gestation but not in the period between days 5-6 and 20-29 Maternal bodyweight gain reduced by 89% in the period between 10-14 days of gestation and by 56% in the period between days 14-20 of gestation Increased incidence of stool variations (18/19 versus 11/19 in controls) Significant reductions in food consumption during the dosing period and increase afterwards until sacrifice **NOAEL maternal toxicity: 100 mg/kg bw/d**	

Table: Summary of the repeated toxicity studies by inhalation route with propiconazole.

Method **Exposure**	**Species** **Strain** **Nº/group** **Dose levels**	**Results**	**Reference**
90-day, subchronic inhalation toxicity study Equivalent or similar to OECD TG 413 6 hours/day 5 days/week	Rat RAIf (SPF) 20 males and 20 females/group 0 (air), 10 mg/m^3 acetone (vehicle control), 21±2, 85±7 or 191±10 mg/m^3 (mean achieved concentration) Nose/head only	<u>21 mg/m^3:</u> Body weight: ↓ by 5.9%, 7.3% and 5.3% females at weeks 4, 8 and 12 respectively <u>85 mg/m^3:</u> Body weight: ↓ by 2.2%, 6.3%, 6.5% and 3.5% in males at weeks 1, 4, 8 and 12 respectively; 4.5% and 4.1% in females at weeks 4 and 8 respectively <u>191 mg/m^3:</u> Body weight: ↓ by 4.8%, 5.3%, 3.5% and 0.5% in males at weeks 1, 4, 8 and 12 respectively; 1.1%, 5.9%, 7.3% and 5.3% in females at weeks 1, 4, 8 and 12 respectively **NOAEC males: 21 mg/m^3** **LOAEC females: 21 mg/m^3**	DAR II A 5.3.3/02

Table: Summary of the repeated toxicity studies by dermal route with propiconazole.

Method **Exposure**	**Species** **Strain** **Nº/group** **Dose levels**	**Results**	**Reference**
21-day study	Rabbit New Zealand White	<u>200 mg/kg bw/d:</u> Slight skin irritation (no differences between intact and abraded skin)	DAR IIA 5.3.3/01

Equivalent or similar to OECD TG 410 Shaved skin of the back covered with a gauze and occlusive dressing intact and abraded skin 6 hours/day 5 days/week	10 males and 10 females/group 0, 200, 1000 and 5000 mg/kg bw/d	<u>1000 mg/kg bw/d:</u> Slight skin irritation (no differences between intact and abraded skin) Clinical observations: From day 4: sedation (10/10 males, 10/10 females). Days 15-21: ruffled fur, tremor, dyspnoea and diarrhoea (number of animals affected not reported) Histopathology: moderate acanthosis and hyperkeratosis of epidermis, chronic inflammatory infiltration in dermis (4/10 males, 4/10 females). Minimal or slight focal acanthosis of epidermis, slight chronic infiltration in dermis (5/10 males) <u>5000 mg/kg bw/d:</u> Slight skin irritation (no differences between intact and abraded skin) Clinical observations: From day 4: ruffled fur (10/10 males, 10/10 females), dyspnoea (10/10 males, 10/10 females), tremor (10/10 males, 10/10 females), ataxia (10/10 males, 10/10 females) and sedation (10/10 males, 10/10 females) Body weight: ↓ by 8.6%, 8.7% and 12.5% on days 12, 15 and 19, respectively, in females Histopathology: marked acanthosis and hyperkeratosis of epidermis, chronic inflammatory infiltration and focal fibrosis in dermis (6/10 males, 9/10 females). Necrosis of epithelium, marked acanthosis and hyperkeratosis of epidermis, focal fibrosis and chronic infiltration in dermis (3/10 males, 1/10 females). Minimal or slight focal acanthosis of epidermis, slight chronic infiltration in dermis (1/10 males) **NOAEL systemic effects = 200 mg/kg bw/d** **LOAEL systemic effects = 1000 mg/kg bw/d**	
Equivalent or similar to OECD TG 410	Rat	<u>10 mg/kg bw/d:</u> No adverse effects observed	dRAR B.6.3.3.1.2

GLP Shaved skin of the back covered with an occlusive dressing 4 weeks Weeks 1-3: 5 days/week Week 4: 7 days/week	Hanlbm:WIST (SPF) 10 males and 10 females/group 0, 10, 100, 1000 mg/kg bw/d	<u>100 mg/kg bw/d:</u> No adverse effects observed <u>1000 mg/kg bw/d:</u> Blood clinical chemistry: protein ↑ 5.6% (F), globulin ↑ 8.2% (F), A/G ratio ↓ 4.5% (F), cholesterol ↑ 28.6% (F), chloride ↓ 2.3% (F) Organ weights: absolute liver weight ↑ 18.9% (M), 14.0% (F), liver weight relative to bw ↑15.1% (M), 9.5% (F) Histopathology: acanthosis (minimal) at application site (8/10 F compared to 1/10 F controls). **NOAEL systemic effects = 100 mg/kg bw/d** **LOAEL systemic effects = 1000 mg/kg bw/d**	

Comparison with the criteria

The three tables above summarise the available information regarding the toxicity exerted by propiconazole after repeated exposures. The vast database contains information about sub-acute toxicity in rat by oral route and in rabbit by dermal inhalation route, sub-chronic toxicity in rat and mouse by oral route, sub-chronic toxicity in rat by inhalation route, sub-chronic and chronic toxicity in dogs by oral route, chronic toxicity in rat and mouse by oral route, a 2-generation reproduction study in rat and three developmental toxicity studies (two in rat and one in rabbit) and draws a toxicity profile where the main target organ is liver, with clinical alterations probably associated to hepatotoxicity, with reductions in bodyweight gain and with clinical symptoms at certain high exposures.

The clinical sings were reported in the teratology studies at 300 and 360 mg/kg bw/d in rat and in the subacute dermal toxicity in rabbit at doses of 1000 and 5000 mg/kg bw/d. RAC notes that Annex 1: 3.9.2.8 of the "Guidance on the Application of the CLP Criteria" excludes clinical observations that have toxicological importance but that do not, by themselves, indicate significant toxicity as effects considered for supporting classification as STOT RE. In addition, it is also remarkable that: i) the effects in teratology studies appear after exposures in the same order of magnitude of the reported LD_{50} and therefore a potential classification based on these clinical effects would cause a double-classification considering the Acute Toxicity Category 4; ii) the effects in sub-chronic dermal studies appear well above the limit for classification as STOT RE category 2 (600 mg/kg bw/d). In conclusion, **RAC does not consider that the clinical effects fulfil the criteria for classification as STOT RE.**

Several studies reported alterations in clinical chemistry that were likely associated to the hepatotoxicity. These alterations were of minor toxicological significance or appeared at concentrations above the limits for warranting classification as STOT RE 2. In addition, Annex 1: 3.9.2.8 of the "Guidance on the Application of the CLP Criteria" describes small changes in clinical biochemistry as example of effects that do not support classification as STOT RE.

Therefore, in the case of propiconazole RAC does not consider the changes in clinical chemistry seen relevant for STOT RE classification.

Reductions in body weight were reported very often in the assessed studies (see the three tables above). The table below summarises the studies reporting these reductions in body weight and the lowest exposure for which such reduction was reported.

Table: Reductions in bodyweight reported after repeated exposures to propiconazole. Data were taken from the studies summarised in the three tables above. Bold text refers to those effects that appear at doses relevant for classification as STOT RE.

Study	Lowest reported dose (mg/kg bw/d) (except in inhalation studies)	Guidance value for STOT RE classification (mg/kg bw/d) (except in inhalation studies)
90-day oral toxicity in rat	460-481	10 ≤ C ≤ 100
2-year chronic toxicity in rat	18-23	1.25 ≤ C ≤ 12.5
2-year chronic toxicity in mouse	344-340	1.25 ≤ C ≤ 12.5
2-generation reproduction toxicity	214-243	4 ≤ C ≤ 40 (assuming 32 weeks of exposure in F_0)
Developmental toxicity in rats	**300**	**90 ≤ C ≤ 900**
Developmental toxicity in rabbits	**250**	**50 ≤ C ≤ 500**
90-day inhalation toxicity in rat	**21 mg/m³**	**20 ≤ C ≤ 200 mg/m³**
Sub-acute dermal toxicity in rat	1000-5000	60 ≤ C ≤ 600

The reductions in bodyweight found in the developmental toxicity in rats were reported concurrently with severe clinical signs and at doses in the same order of magnitude as the LD_{50} and are therefore not relevant for classification purposes. The reductions in the bodyweight in the developmental study in rabbits appear at concentration within the range of the classification criteria for Category 2. However, in this study the reductions in body weight were seen during the administration period and there were no statistically significant differences between the corrected body weight of all groups at the end of the study. The effects reported in the sub-chronic inhalation study could also be potentially relevant for classification in Category 2. However, in this study the effects in body weight were of relatively low severity and not dose-related, which significantly limits their toxicological importance. In addition, the already above stated Annex 1: 3.9.2.8 of the "Guidance on the Application of the CLP Criteria" recognises small changes in bodyweight gain as effects that do not fulfil the criteria for STOT RE classification. Therefore, RAC does not consider these changes in bodyweight as relevant for STOT RE classification of propiconazole.

Hepatotoxicity was consistently noted in most of the repeated toxicity studies. The main reported hepatic effects were liver weight increases, hypertrophy, vacuolation, necrosis and mineralization. The table below summarises the studies reporting hepatotoxicity and the lowest exposure for which such reduction was reported.

Table: Hepatotoxicity reported after repeated exposures to propiconazole. Bold text refers to those effects that appear at doses relevant for classification as STOT RE.

Study	Lowest reported dose (mg/kg bw/d)	Guidance value for STOT RE classification (mg/kg bw/d)
28-day oral toxicity in rat	**150**	**30 ≤ C ≤ 300**
90-day oral toxicity in rat	461/481	10 ≤ C ≤ 100
90-day oral toxicity in mouse (2 studies)	**65-71-85**	**10 ≤ C ≤ 100**
2-year oral toxicity in rat	96-130	1.25 ≤ C ≤ 12.5
2-year oral toxicity in mouse	49-55	1.25 ≤ C ≤ 12.5
18-month oral toxicity in mouse	59	2.2 ≤ C ≤ 22
2-generation reproduction toxicity in rat	**44-49**	**4 ≤ C ≤ 40 (assuming 32 weeks of exposure in F_0)**

Four of the different studies (90-day and 2-year oral toxicity studies in rat, and 2-year and 18-month oral toxicity studies in mouse) showed that the hepatotoxicity appeared at doses well above the respective limit dose for warranting classification as STOT RE 2. Therefore RAC does not consider these studies relevant for classification. However, the other three studies (28-day in rat, 90-day oral toxicity studies in mice and 2-generation reproduction toxicity in rat) showed hepatotoxicity at doses either warranting classification as STOT RE Category 2 or on the border for classification.

Annex 1: 3.9.2.8 of the "Guidance on the Application of the CLP Criteria" states that adverse effects relevant for classification would have to be toxicologically significant and affect the function or morphology of a tissue/organ. However, the effects reported at 150 mg propiconazole/mg bw/d in the 28-day oral toxicity study in rat were described as minimal hypertrophy of hepatocytes and small focus organising necrosis in liver parenchyma, while the dose showing moderate hypertrophy and multiple areas of necrosis was 450 mg/kg bw/d, and hence above the limit for classification.

The two different 90-day oral toxicity studies in mouse yielded results compatible with STOT RE classification due to liver toxicity. However, the severity of hepatocellular necrosis was observed to be slight or very slight in all animals except in two individuals where was scored as moderate.

The 2-generation reproduction toxicity study in rat reported at 44-49 mg/kg bw/d liver hypertrophy in F_0 and F_1 in males affecting 93 and 33% of examined males and 50% of F_1 females. These alterations were not significant in other generations. The incidence and relevance of these alterations were not considered by RAC as toxicologically relevant for warranting classification. In the same study, liver hypertrophy was consistently reported in four different generations in almost 100% of male and female, although in this case these effects were found at 215-243 mg/kg bw/d, which is above the limits for warranting classification as STOT RE category 2.

In conclusion, RAC notes that the hepatotoxicity associated with repeated exposure of propiconazole either appears at concentrations above the cut-off values for warranting classification or when appearing below these limits, is seen with a severity and incidence not considered indicative of toxicologically relevant disturbances. Therefore RAC supports the DS's proposal and **does not propose classification of propiconazole for STOT RE.**

4.8 Germ cell mutagenicity (Mutagenicity)

The data on mutagenicity is included here as supporting evidence for the carcinogenicity endpoint. No classification is discussed or proposed for this endpoint for propiconazole.

Table 20: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results and remarks	Reference
In vitro		
Bacterial reverse mutation assay (Ames test; gene mutation) S. typhimurium TA 98, TA 100, TA 1535 and TA 1537 (with and without S9-mix) E. coli WP2pKM101and WP2uvrApKM101 (with and without S9-mix) Test concentrations: 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate Purity: 94.1% GLP Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Test)	Evaluation of results: Negative with and without metabolic activation. Cytotoxicity: Yes. Reduction in the number of revertants for TA1535, TA1537, TA98, TA100 and WP2pKM101 with/without S9 mix. Reduction in number of revertants Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	dRAR B.6.4.1.1
Bacterial reverse mutation assay (Ames test; gene mutation) S. typhimurium TA 98, TA 100, TA 1535, TA 1537 and TA 1538 (with and without mouse S9-mix) Test concentrations: 20, 80, 320, 1280 and 5120 µg/plate Purity: 90.7% Similar to OECD Guideline 471 (Bacterial Reverse Mutation Test)	Evaluation of results: Negative with and without metabolic activation Cytotoxicity: Yes; Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	DAR IIA 5.4.1/01
Mammalian cell gene mutation assay (L5178Y cells) 5, 10, 30, 40, 50, 60, 70 and 80 ug/ml (with S9-mix). 10, 20, 30, 50, 70, 80 and 90ug/ml (without S9-mix) Purity: 94.1% GLP	Evaluation of results: Negative with and without metabolic activation Test results: The top doses used were limited by toxicity. No substantial or reproducible concentration-dependent increase of the mutation frequency was observed in any of the three experiments conducted. Vehicle controls valid: Yes; Positive controls valid: Yes	dRAR B.6.4.1.2

Method	Results and remarks	Reference
Equivalent to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)		
Mammalian cell gene mutation assay (L5178Y cells) Test concentrations: 7.81, 15.62, 31.25, 62.50, 125.0 µg/mL (with and without S9-mix)Purity: 90.7% Equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) equivalent or similar to EU Method B.17 (Mutagenicity - In Vitro Mammalian Cell Gene Mutation Test) equivalent or similar to EPA OPPTS 870.5300 - In vitro Mammalian Cell Gene Mutation Test	Evaluation of results:Negative with and without metabolic activation Test results: Negative for mouse lymphoma L5178Y cells with and without metabolic activation Cytotoxicity: Yes (at 125 µg/mL); Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	DAR IIA 5.4.1/02
In vitro mammalian cell cytogenetic test (Human lymphocytes; chromosome aberration) Test concentrations: 11.25, 45, 90 and 180 µg/mL (with S9-mix) 11.25, 22.5, and 180 µg/mL (without S9-mix) Purity: 89.7% Equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	Evaluation of results: Negative with and without metabolic activation Test results: Negative for lymphocytes: human with and without metabolic activation Cytotoxicity: Yes (at 180 µg/mL (the highest dose tested)) Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	DAR IIA 5.4.1/04
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (Rat hepatocytes) Test concentrations: 0.67, 3.34, 16.69 and 83.47 µg/mL (Exogenous metabolic activation not applied) Purity: 90.7% Equivalent or similar to OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)	Evaluation of results: Negative Test results:Top dose limited by toxicity observed in a preliminary experiment. Silver grains per nucleus in cells treated with propiconazole compared with controls not markedly increased. Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	DAR IIA 5.4.2/02
In vitro mammalian cell transformation assay (Mouse fibroblasts; BALB/3T3) Test concentrations: 1.16, 2.31, 4.63, 9.25 and 18.5 µg/mL	Evaluation of results: Negative Test results: Negative for mouse fibroblasts Cytotoxicity: based on a preliminary test to cause a reduction in colony forming ability of ca 25% Vehicle controls valid: Yes; Negative controls valid: Yes;	DAR IIA 5.4.1/03 Not acceptable

Method	Results and remarks	Reference
(Exogenous metabolic activation not applied) Purity: 90.7% Equivalent or similar to EU Method B.21 (In Vitro Mammalian Cell Transformation Test)	Positive controls valid: Yes	
In vivo		
Dominant lethal assay Mouse: Tif:MAGf (20 males for each dose group, mated with untreated females) Purity 90.0% Test doses: 165 and 495 mg/kg Females autopsied on the 14th day of pregnancy Equivalent or similar to EPA OPPTS 870.5450 (Rodent Dominant Lethal Assay)	Evaluation of results: Negative Test results: Negative (no significant difference from controls in number of implantations or in embryonic deaths); Toxicity: Yes (clinical signs post dosing in males at 495 mg/kg) ; Vehicle controls valid: Yes; Negative controls valid: Not applicable; Positive controls valid: Yes	DAR IIA 5.4.3/01
Micronucleus assay (Bone marrow) Mouse: Ico:CD1(CRL)) (5 males and 5 females for each dose group) Doses: 80, 160, 320 mg/kg). Sampling times: 24h, and 48h (top dose only). Purity: 92.4% GLP OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) EPA OPPTS 870.5395 (In Vivo Mammalian Cytogenics Tests: Erythrocyte Micronucleus Assay) EU Method B.12 (Mutagenicity - In Vivo Mammalian Erythrocyte Micronucleus Test) MITI (Japan) 62 Notification of Basic Industries Bureau, 1987	Evaluation of results: Negative Test results: No significant increase in micronucleated polychromatic erythrocytes over controls. Genotoxicity: Negative (male/female); Toxicity: Yes (In the preliminary toxicity assay, toxicity seen at 800 and 2000 mg/kg but not at 320 mg/kg. In the micronucleus test toxicity seen at 320 mg/kg. Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	DAR IIA 5.4.2/03
Micronucleus assay (bone marrow) Hamster, Chinese (8 males and 8 females for each dose group) Test doses: 0, 307.5, 615.0, 1230.0 mg/kg Sampling times: 24h. The top dose was also sampled after 16, 24 and 48h in a separate experiment. Purity 91.1% OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: Negative Test results: No significant increase in micronucleated polychromatic erythrocytes over controls. Genotoxicity: negative (male/female); toxicity: yes (In preliminary study, 4/4, 1/4, 1/4, 1/4, and 1/4 mortalities at dose levels of 5000, 3000, 2400, 1920 and 1536 mg/kg) Vehicle controls valid: Yes; Negative controls valid: Yes; Positive controls valid: Yes	DAR IIA 5.4.2/01

Method	Results and remarks	Reference
EU Method B.12 (Mutagenicity - In Vivo Mammalian Erythrocyte Micronucleus Test)		

4.8.1 Non-human information

4.8.2 Summary and discussion of mutagenicity

The mutagenicity of propiconazole has been examined *in vitro* in 2 bacterial reverse mutation assays; a chromosomal aberration assay in human lymphocytes; two mammalian cell gene mutation assays in L5178Y cells; a DNA damage/repair assay in isolated rat liver cells and a mammalian cell transformation assay. *In vivo*, the mutagenicity potential of propiconazole has been investigated in a mouse dominant lethal assay and in 2 bone marrow micronucleus assays (mouse and hamster). No evidence of mutagenicity of propiconazole was observed. The overall conclusion is that propiconazole is not genotoxic.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

This hazard was not reviewed in the CLH-report.

Comments received during public consultation

One international non-governmental organization submitted a comment alerting about a few open publications on mutagenicity of propiconazole that were not included in the assessment of carcinogenicity by the DS. Similar comments were also submitted by the same international non-governmental organization in the carcinogenicity section. The DS replied that mutagenicity was included in the report only as supporting evidence. Please, see the DS's answer to this comment in the section of carcinogenicity.

Assessment and comparison with the classification criteria

Not applicable because this hazard was not reviewed in the CLH report.

4.9 Carcinogenicity

There is one carcinogenicity study available in the rat and two carcinogenicity studies available in the mouse. The liver pathology of the first mouse study was subsequently re-examined (IIA 5.5/04). In addition, a reference study was conducted to determine the spontaneous liver tumour profiles in untreated mice.

Table 21: Summary table of relevant carcinogenicity studies

Method **Species** **Strain** **No/sex/group**	**Doses** **Route** **Exposure period**	**Results and remarks**	**Reference**
2 year chronic toxicity/ carcinogenicity OECD 453 (1981), GLP Rat, Sprague Dawley CD 80 males and 80 females Acceptable	Oral, in diet Purity:87.2-91.9% 0, 100, 500, 2500 ppm. Doses 0, 3.60, 18.10, 96.46 mg/kg/day (males); 0, 4.57, 23.32, 130.63 mg/kg/day (females) Continuous treatment over 2 years	**No treatment-related neoplastic findings.** **NOAEL overall: 100 ppm** (males 3.60 mg/kg bw/day, females 4.57 mg/kg bw/day) 500 ppm (males 18.10 mg/kg bw/day, females 23.32 mg/kg bw/day): Reduced body weight gain and food utilization in females and lower adrenal weights in males . 2500 ppm (males 96.46 mg/kg bw/day, females 130.63 mg/kg bw/day): Reduced body weight gain and food consumption in both sexes. Increased liver weights in both sexes and increased incidence of foci of enlarged liver cells in females. Lower adrenal weights in both sexes.	DAR IIA 5.5/02 Key Study
2 year carcinogenicity OECD 451 (1981), GLP Mouse CD-1 64 males and 64 females Acceptable	Oral, in diet Purity: 87.2-91.9% 0, 100, 500 and 2500 ppm Doses 0, 10.04, 49.39, 344.27 mg/kg/day (males); 0, 10.79, 55.60, 340.26 mg/kg/day (females) Continuous treatment over 2 years	**NOAEL carcinogenicity: 500 ppm**, (49.39 mg/kg bw/day for males and 55.60 mg/kg bw/day for females) based on increased incidence of hepatocellular adenoma and carcinoma in males at 2500 ppm (344.27 mg/kg bw/day). **NOAEL overall: 100 ppm** (10.04 mg/kg bw/day for males and 10.79 mg/kg bw/day for females) 500 ppm (49.39/55.60 mg/kg bw/day for males/females): Increased liver weights and increased incidence and severity of hepatocyte enlargement in males. Reduced body weight gains in both sexes. 2500 ppm (344.27/340.26 mg/kg bw/day for males/females): Slight increase of mortality in males during first 6 months. Reduced body weights in males and reduced weight gains in both sexes. Hepatotoxic signs in both sexes including increased liver weights, increased incidences of centrilobular hepatocyte enlargement and hepatocyte vacuolation. In males also chronic inflammation, pigmented Kupffer cells, hepatocyte necrosis (at interim) and increased serum levels of hepatic enzymes were observed.	DAR IIA 5.5/03 DAR IIA 5.5/04 Key Study

<table>
<tr><th>Method
Species
Strain
No/sex/group</th><th>Doses
Route
Exposure period</th><th>Results and remarks</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td>18 Month Study in CD-1 male mice

OECD 451 (1981), GLP

Mouse Crl: CD-l (ICR) BR

80 males/group

<u>Acceptable</u></td><td>Oral, in diet

Purity: 92.4 %

0, 100, 500 and 850 ppm

Doses 0, 11.0, 59.0, 107.6 mg/kg/day

Continuous treatment over 18 months</td><td><u>NOAEL carcinogenicity</u>: 500 ppm based on increased incidence of hepatocellular adenoma (20% vs. 2% in controls) at 850 ppm (59 mg/bw kg/day).

<u>NOAEL overall:</u> 100 ppm (11.0 mg/kg bw/day) based on reduced body weight gains, increased liver weights, reduced plasma cholesterol levels at 500 and 850 ppm, and increased sorbitol dehydrogenase levels at 850 ppm. Increased incidence of hepatocellular hypertrophy at 500 and 850 ppm. Enlarged livers, increased incidences of masses and nodules, foci of cellular change and Kuppfer cell pigmentation at 850 ppm. Slightly increased incidence of hepatocyte necrosis at 850 ppm after 9 weeks treatment.</td><td>DAR IIA 5.5/05</td></tr>
<tr><td>18 Month Reference Study in CD-1 mice

OECD 451 (1981)

Mouse ICOCD-1 (Crl)

5 groups of 80 males and 80 females (50/sex main study, 10/sex for haematological investigations 10/sex for clinical chemistry and urinalysis investigations and 10/sex for interim sacrifice at 13 weeks).</td><td>Fed control diets for 18 months</td><td>Incidences of hepatocellular tumours in CD-1 males varied 14-30% (n=5 studies) with 3/5 ≥ 24%. No tumours were observed in females.

Intergroup comparison of microscopic findings in the livers of control CD-1 males
<table>
<tr><th>Finding</th><th colspan="5">Control Groups</th></tr>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th></tr>
<tr><td>Number examined</td><td>50</td><td>50</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td>Hepatocellular adenoma</td><td>9</td><td>7</td><td>3</td><td>4</td><td>7</td></tr>
<tr><td>Hepatocellular adenocarcinoma</td><td>6</td><td>8</td><td>5</td><td>4</td><td>7</td></tr>
<tr><td>Combined - Hepatocellular adenoma + adenocarcinoma</td><td>15</td><td>12</td><td>7</td><td>7</td><td>13</td></tr>
</table></td><td>DAR IIA 5.5/05</td></tr>
</table>

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

In a chronic toxicity and carcinogenicity study in rat (DAR IIA 5.5/02), propiconazole (purity in two independent analyses 87.2 and 91.9%) was administered to groups of 80 male and 80 female

Sprague-Dawley CD rats in diet at concentrations of 0, 100, 500 and 2500 ppm for up to approximately 2 years (107/109 weeks for males/females, respectively). The doses corresponded to an achieved intake of 0, 3.60, 18.10 and 96.46 mg/kg/day for males and 0, 4.57, 23.32 and 130.63 mg/kg/day for females. The rats were 4 weeks old at the onset of the test, weighing 160-163 g (males) and 115-118 g (females). 50 animals per sex and dose group were allocated primarily for tumorigenic evaluation, 10 animals/sex/dose group for haematological examinations, 10 animals/sex/dose group for blood chemistry and urinanalysis, and 10 animals/sex/dose group for interim sacrifice after 12 months of treatment. Mortality was checked twice per day. Clinical signs were observed daily during the first 4 weeks and weekly thereafter. All rats were subjected to a detailed macroscopic examination with organ weight analysis and organs were preserved and processed for histopathological examination.

Mortality rates at the end of the study were as follows: 38, 39, 40 and 31% for males and 53, 45, 45 and 33% in females of dose groups 0, 100, 500 and 2500 ppm, respectively. Food intake was significantly reduced, up 12% lower than controls, high dose (2500 ppm) females during the whole treatment period. This was also apparent to lesser extent in high dose males (up to 6% lower than controls). Over the first 26 weeks of treatment, inferior efficiencies of food utilisation were recorded for both sexes at high dose, and for females at intermediate (500 ppm) dose when compared with controls. This was associated with reduced body weight gains in these groups particularly during first year of the treatment (16% and 35% lower than controls in high dose males and females respectively). No other treatment-related clinical signs were observed during the study. Yet, clinical signs were observed only weekly, instead of daily, during major part of the study.

Significantly higher serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels were observed in females in all dose groups during week 26. Later examinations (weeks 52, 78 and 104) showed no elevations in these enzymes. Higher urea nitrogen levels were recorded in high dose females during weeks 26, 33 and 52, and also in males of the high dose group during week 78. Significantly lower blood glucose was observed in high dose females and males, and intermediate dose females, during weeks 26, 52, and 78 of the treatment. Slight inconsistent reductions were observed in packed cell volume, haemoglobin, white blood cell and neutrophil counts in high dose animals during some weeks in comparison to controls.

Liver weights were significantly increased in both sexes of high dose group in interim and at the end of the study (up to 16 % and 19% higher than controls in males and females, respectively). In high dose females this was associated with an increased incidence in foci of enlarged liver cells (13/71 vs. 1/70 in controls). Adrenal weights were significantly lower in both sexes of high dose group and in males of the intermediate group at the end of the study. Significantly lower kidney weights were recorded in high dose females and slightly, but not significantly, lower kidney weights for high dose males. Histopathological examinations revealed no treatment-related changes in adrenal or kidney. The incidence and distribution of tumours showed no treatment-related findings.

In conclusion, significant decrease in body weight gain, slight haematological effects, together with increases in urea nitrogen levels, were observed. These were consistent with similar results shown in the 90-day oral rat study with higher doses up to 6000 ppm (DAR IIA 5.3.2/01). Increased liver weights in both sexes and increased incidence of foci of enlarged liver cells in females were observed at 2500 ppm (males 96.46 mg/kg bw/day, females 130.63 mg/kg bw/day). In addition, the observed increases in hepatic enzyme levels indicate that liver is a target organ for propiconazole in rats. Lower adrenal weights were observed in both sexes at 2500 ppm and in males at 500 ppm. There was no evidence of treatment-related tumourgenesis. The NOAEL was 100 ppm, equivalent to 3.60 mg/kg bw/day for males and 4.57 mg/kg bw/day for females.

In a carcinogenicity study in mouse (DAR IIA 5.5/03), CD-1 mice in groups of 64 males and 64 females, were exposed to 0, 100, 500 or 2500 ppm propiconazole (purity in two independent analyses 87.2 and 91.9%) in diet for 2 years (102 and 104 weeks, males and females respectively), with interim sacrifices at 52 weeks (DAR IIA 5.5/03). The time weighted average daily intakes of propiconazole were 0, 10.0, 49.4 and 344.3 mg/kg bw/day for males and 0, 10.8, 55.6 and 340.3 mg/kg bw/day for females. The mice were 4 weeks old at the start of the experiment. 10 animals/sex/dose were allocated to haematological examinations, 10 animals/sex/dose to clinical chemistry examinations and 12 animals/sex/dose to interim sacrifice after 52 weeks of treatment. Mortality was checked twice per day. Clinical signs were observed twice per day for the first four weeks and weekly thereafter. Body weights and food consumption were recorded weekly. Haematology and clinical chemistry (blood and urine) analyses were carried out after 1 year and at termination. All animals were subjected to gross pathological examination. Selected organs were weighed and a range of tissues were taken for microscopial examination (animals sacrificed in interim and termination).

Mortality was high during the study; for males 41, 52, 50 and 64%, and for females 38, 31, 45 and 31%, in dose groups of 0, 100, 500 and 2500 ppm, respectively (Table 22). During first 26 weeks, mortality was slightly increased in the high dose males (5 vs 0 deaths in controls). However, there were no significant pathological findings and survival of this group remained similar to controls thereafter. The highest mortality in all groups occurred during the second year of the treatment.

Table 22: Mortality during carcinogenicy study in mouse.

Deaths during weeks/no. mice at the beginning of the study	**Dietary concentration of propiconazole**							
	Males				**Females**			
	0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
1-26	0/64	1/64	1/64	**5/64***	1/64	1/64	1/64	0/64
27-52	2/64	5/63	5/63	5/59	3/63	3/61	3/61	1/63
53-78[a]	11/51	11/47	8/47	12/45	6/48	3/47	7/47	3/50
79-104[a]	16/40	16/36	18/39	19/33	14/42	13/44	18/40	16/47
1-104 (%)	29/64 (41)	33/64 (52)	32/64 (50)	41/64 (64)	24/64 (38)	20/64 (31)	29/64 (45)	20/64 (31)

* $p<0.05$ [a] The numbers exclude mice sacrificed at interim

Food consumption of high dose (2500 ppm) males was significantly increased throughout the treatment. Despite this, the body weights and cumulative weight gain of this group remained significantly reduced throughout the study, indicating reduced food utilization (**Table 23**). The same effect was apparent, but less marked in high dose females. Absolute body weights of high dose males remained 12-20 % lower than controls throughout the study, suggesting that maximum tolerated dose was exceeded. Cumulative weight gains of intermediate dose (500 ppm) males and females were reduced at the beginning of the study but recovered thereafter (**Table 23**).

Table 23: Mean food intakes, body weights and cumulative body weight gains in carcinogenicity study in mouse.

	Dietary concentration of propiconazole							
	Males				Females			
	0 ppm	100 ppm	500 ppm	2500 ppm	0 ppm	100 ppm	500 ppm	2500 ppm
Food intake (g/mouse) week								
1-20	671	667	673	**802*** **	592	597	594	**688*** **
21-52	976	1018	993	**1111** **	856	840	834	823
53-78[a]	796	826	797	**961*** **	729	691	697	774
79-104	796	783	760	**1099*** **	721	668	693	703
Body weight (g) [a] week								
0	27.09	27.41	27.84*	27.28	21.28	21.70	20.56**	20.30***
13	41.88	41.38	40.80	**35.87*** ** (14.4%↓)	29.76	30.73	30.32	**27.70*** ** (7%↓)
26	45.47	44.56	43.56	**39.47*** ** (13.2%↓)	33.48	34.59	33.78	**30.66*** ** (8.5%↓)
52	47.39	46.67	46.38	**41.39*** ** (12.7%↓)	35.82	37.45	36.08	**32.52*** ** (9.3%↓)
78	51.60	49.69	50.32	**41.23*** ** (20%↓)	37.24	41.04**	39.03	**33.85** ** (9.2%↓)
104	48.83	47.05	47.86	**40.86*** ** (16.4%↓)	36.29	38.53	37.71	**32.56** ** (10.3%↓)
Cumulative weight gain (g) [a]								
weeks 0-13	14.78	13.97	**12.95*** **	**8.55*** ** (42.2%↓)	8.44	9.03	**9.70** **	**7.48*** (11.4%↓)
0-26	18.38	17.19	**15.70*** **	**12.02*** **	12.21	12.84	13.25	**10.44** **
0-52	20.29	19.29	**18.45***	**13.87*** ** (32%↓)	14.63	15.57	15.62	**12.30** ** (16%↓)
0-78	24.60	22.19	22.41	**13.73*** **	16.24	19.24*	18.53	**13.83***
0-104	21.79	19.80	19.71	**13.07*** ** 40%↓	15.11	16.61	17.71*	**12.16** ** 19%↓

* p<0.05, ** p <0.01, *** p<0.001 [a] Supplemental statistical analyses

Slightly lower haemoglobin levels in comparison to controls were recorded in high dose males after one year of treatment, but no difference was apparent at the end of the study. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased among high dose males after one year and in termination. At the end of the study, alkaline phosphatase (ALP) was also significantly increased in high dose males. Some indication of increase in ALT and AST was also noted among females of the high dose group, but the difference to controls was not

statistically significant. Slightly lower serum cholesterol levels were observed in high dose males and females after one year, although in females the difference to controls was not statistically significant. At termination cholesterol levels were lower in high dose females compared to controls.

Significantly increased liver weights were observed in high dose males and females at interim and in terminal sacrifice (Table 24). At interim liver weights were significantly increased also in intermediate dose (500 ppm) males. Moreover, increased liver masses were observed in two intermediate dose males and in four high dose males (zero incidence in controls) at interim, and increased incidence of liver masses and/or enlarged livers was observed among intermediate and high dose males, and among high dose females in terminal sacrifice.

Table 24: Mean liver weights in carcinogenicity study in mouse

	Dietary concentration of propiconazole							
	Males				**Females**			
	0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
Interim								
absolute (g)	2.25 [a]	2.46	**2.78***	**4.37*****	1.75	1.65	1.68	**2.42*****
relative (/body weight)	0.051 [a]	0.054	**0.063***	**0.112*****	0.049	0.049	0.050	**0.081*****
Terminal								
absolute (g)	3.24 [a]	2.95	3.43	**7.36*****	2.00 [a]	1.93	1.86	**3.01*****
relative (/body weight)	0.069	0.067	0.073	**0.182*****	0.056 [a]	0.052	0.050	**0.091*****

[a] values logarithmically transformed to stabilize variances, * p<0.05, ** p<0.01, ***p<0.001. Supplemental statistical analyses.

Histopathological analysis revealed signs of hepatotoxicity in both sexes and increased liver tumour incidence primarily in high dose males. No treatment-related effect was seen in the incidence and distribution of other tumour types, nor in organ weights or tissue histology.To confirm the original diagnosis, the observed tumour response and non-neoplastic changes of the liver were subsequently re-examined (DAR IIA 5.5/04). The morphology and biological behaviour of the liver tumours observed were evaluated according to new (at that time), diagnostic nomenclature and morphologic criteria. All liver specimens from males and females were examined for histopathological changes including neoplastic and non-neoplastic lesions. Proliferative hepatocellular lesions were classified as either foci of cellular alteration, benign (hepatocellular adenoma) or malignant (hepatocellular carcinoma) using the criteria published by the U.S. National Toxicology Program. The hepatocellular carcinomas were classified by degree of differentiation. In addition, all specimens of lung from males were examined for the presence of pulmonary metastasis from malignant hepatocellular neoplasms. Since re-examination largely confirmed the findings of the original study, only the findings of re-examination are included in detail below.

Histological observations pointing to hepatotoxicity were observed in males at 500 and 2500 ppm and in females at 2500 ppm (**Table 25**). The hepatotoxic effects observed in high dose males were: centrilobular hepatocyte enlargement, hepatocellular vacuolation, chronic inflammation, hepatocyte necrosis, and accumulation of pigment within phagocytic (Kupffer) cells of the liver. According to original study, hepatocyte enlargement was associated with dilated and/or congested liver sinusoids. In addition, the incidence of eosinophilic cell foci was higher in high dose males than in controls,

although not statistically significantly. In high dose females significantly increased incidences of hepatocyte enlargement and hepatocellular vacuolation were observed. Increased centrilobular hepatocellular enlargement was the only hepatotoxic sign in the 500 ppm male mice group. Histologic findings correlated closely with reported changes in hepatic enzyme levels (ALT, AST, and ALP), increased liver weights and macroscopic findings of liver masses and enlarged livers.

Table 25: Non-neoplastic microscopy changes in the liver in carcinogenicity study in mouse

	Dietary concentration of propiconazole							
	Males				**Females**			
	0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
Interim sacrifice								
Number of examined	11	11	11	9	12	11	11	12
Hepatocyte Enlargement	2	4	**8***	**9*****	0	0	0	**7****
Hepatocyte Vacuolation	6	2	4	2	3	**8***	5	**10***
Hepatocyte Necrosis	0	0	0	**4***	0	0	0	0
Inflammatory cell Infiltration Chronic	1	0	2	**6***	0	0	0	0
Pigmented Kupffer Cells	0	0	0	3	0	0	0	0
Basophilic Cell Focus	0	1	0	0	0	0	0	0
Eosinophilic Cell Focus	0	0	0	0	0	0	0	0
Terminal Sacrifice and decedents								
Number of examined	53	53	51	55	52	53	53	52
Hepatocyte Enlargement	12	6	**31*****	**45*****	0	0	0	**36*****
Hepatocyte Vacuolation	7	5	7	**19***	14	11	17	**29****
Hepatocyte Necrosis	5	2	4	3	3	5	2	2
Inflammatory cell Infiltration Chronic	30	26	26	38	30	26	17*	21
Pigmented Kupffer Cells	7	8	8	**37*****	10	11	5	10
Basophilic Cell Focus	5	5	7	1	0	2	2	0
Eosinophilic Cell Focus	1	1	5	6	1	1	0	4
Total								
Number of examined	64	64	62	64	64	64	64	64
Hepatocyte Enlargement	14	10	**39*****	**54*****	0	0	0	**43*****
Hepatocyte Vacuolation	13	7	11	21	17	19	22	**39*****
Hepatocyte Necrosis	5	2	4	7	3	5	2	2
Inflammatory cell Infiltration Chronic	31	26	28	**44***	30	26	**17***	21
Pigmented Kupffer Cells	7	8	8	**40*****	10	11	5	10
Basophilic Cell Focus	5	6	7	1	0	2	2	0
Eosinophilic Cell Focus	1	1	5	6	1	1	0	4

Incidences are based on re-examination of liver micropathology (DAR IIA 5.5/04).
*p<0.05;**p<0.01; ***p<0.001, pairwise Fisher's Exact Test.

At interim sacrifice (53 weeks) number of males with hepatocellular adenomas was slightly increased in 500 ppm group and number of males with adenomas and/or carcinomas was slightly increased in high dose group (Table 26**)**. All carcinomas were well differentiated and there was no evidence of pulmonary metastasis. There was no indication of neoplasia among the females at interim sacrifice.

For the terminal sacrifice (104 weeks) and decedent animals, the incidences of adenomas and adenomas and/or carcinomas were significantly higher in high dose males than in controls (40% vs. 21% in controls and 80% vs. 51% in controls, respectively, Table 26). The majority of this response was associated with an increased number of hepatocellular adenomas in high dose males. The slight increase in carcinomas was due to an increase in the number of well differentiated hepatocellular carcinomas relative to the control group. The incidences of moderately well and poorly differentiated carcinomas in treated groups were similar to controls. One metastatic carcinoma (pulmonary metastases) was observed in each of the treated groups compared to zero incidence in control group. Number of adenomas and/or carcinomas was slightly, but not significantly, higher in high dose females than in controls (9 vs. 6 in controls, number of mice with adenoma and/or carcinoma). No other indication of neoplasia was observed in females.

The total incidences of adenomas, carcinomas and of adenomas and/or carcinomas in males showed positive linear trend with dose, when all groups were evaluated (method by Peto *et al* 1980). When high dose group was excluded from the analysis, there was no evidence of linear trend. For the respective total incidences in females, the linear trend with dose was not significant ($p > 0.197$).

The tumours were considered to contribute to higher mortality of high dose males (64 % over 104 weeks compared to 41%, 52% and 50%, at 0, 100 and 500 ppm, respectively), since two high dose males were sacrificed due to consequences of liver tumours during last year of the treatment. However, there was no significant difference in the morphologic appearance or biological behaviour of the carcinomas observed in the control as compared to the treated groups. No treatment-related effect was seen in the incidence and distribution of other tumour types.

Table 26: Neoplastic microscopy changes in carcinogenicity study in mouse

	Dietary concentration of propiconazole							
	Males				Females			
	0 ppm	100 ppm	500 ppm	2500 ppm	0 ppm	100 ppm	500 ppm	2500 ppm
Interim sacrifice								
No. animals examined	11	11	11	9	12	11	11	12
Adenoma [a]	1/1	0/0	4/4	4/3	0/0	0/0	0/0	0/0
Carcinoma well differentiated [a]	0/0	0/0	0/0	3/3	0/0	0/0	0/0	0/0
No. of mice with only adenoma	1	0	4	1	0	0	0	0
No. mice with at least one carcinoma	0	0	0	3	0	0	0	0
Adenoma + carcinoma	1	0	4	4	0	0	0	0
Terminal sacrifice and decedents								
No.examined	53	53	51	55	52	53	53	52
Adenoma [a]	25/18	18/11	20/15	68/35	6/5	0/0	2/2	13/8
Carcinoma well differentiated [a] moderately well differentiated [a] poorly differentiated [a]	 10/10 4/4 3/3	 6/5 4/3 2/2	 5/5 9/7 3/3	 23/17 7/6 3/2	 1/1 0/0 0/0	 0/0 1/1 0/0	 0/0 0/0 0/0	 2/2 1/1 0/0
No. mice with only adenoma	11 (21%)	7	9	**22*** (40%)	5	0	2	6
No. mice with at least one carcinoma	16	9	13	22	1	1	0	3
Adenoma + carcinoma	27 (51%)	16	22	**44**** (80%)	6	1	2	9
Total								
No.examined	64	64	62	64	64	64	64	64
No. mice with only adenoma	12[b]	7	13	**23***	5	0	2	6
No. mice with at least one carcinoma	16[b] (25%)	9	13	25 (39%)	1	1	0	3
Adenoma + carcinoma	28[b] (44%)	16	26	**48***** (75%)	6	1	2	9

Incidences are based on re-examination of liver micropathology (DAR IIA 5.5/04).

[a] Total count of tumours/no. mice with tumours * p≤0.05, ** p≤0.01, *** p≤0.001, pairwise Fisher' Exact Test.

[b] p<0.05, positive trend by Peto Trend Test (Groups 1-4), for total animals. For Males: P-values for linear trend = 0.0008 (adenomas), 0.0056 (carcinomas), <0.0001 (adenomas + carcinomas). P-values for linear trend including groups 1 to 3 = 0.3519 (adenomas), 0.6796 (carcinomas), 0.5338 (adenomas + carcinomas). For Females: P-values for linear trend >0.197 (Groups 1-4).

In conclusion, in males the highest dose (2500 ppm) was in excess of a maximum tolerated dose (MTD), based on increased mortality (64% compared to 41% in controls), decreases in absolute body weight (13-20% lower than controls), body weight gains (42% lower than controls), and evidence of hepatotoxicity. In females dosed with 2500 ppm, MTD was exceeded to a lower extent (absolute bw 7-10% and bw gain 19% lower than controls). At the end of the study (104 weeks) increased incidence of liver tumours was observed in high dose males (adenoma and/or carcinoma 80% vs. 51% in controls). The majority of this response was associated with an increased number of hepatocellular adenomas and the slight increase in carcinomas was primarily due to an increase in the number of well differentiated hepatocellular carcinomas. Signs of hepatotoxicity were observed also in high dose females, but there were no significant increases in liver tumour incidences. Evidence of hepatotoxicity was observed in males also at the intermediate dose (500 ppm). The NOAEL was 100 ppm, corresponding to 10.0 mg/kg bw/day propiconazole in males and 10.8 mg/kg bw/day in females.

18-Months carcinogenicity study in mice (DAR IIA 5.5/05) was conducted in response to U.S Environmental protection agency´s (US EPA) requirement for additional mouse oncogenicity study since the high dose (2500 ppm) in the first study (IIA 5.5./04) was considered to be excessively toxic (US EPA, 1992). Groups of 80 CD-1 (ICR) BR male mice were administered diets containing propiconazole (purity 92.4%) at concentrations of 0, 100, 500 and 850 ppm for up to 18 months. After correction for determined dietary concentrations the calculated average intakes of propiconazole were 0, 11.0, 59.0 and 107.6 mg/kg bw/d. There were 4 main groups (including untreated control) each consisting of 50 mice (terminal sacrifice). To each main group was attached a satellite group consisting of 30 animals; 10 were used for blood clinical chemistry; 10 were designated for interim sacrifice at 9 weeks and 10 animals for interim sacrifice at 53 weeks. Clinical signs and mortalities were checked daily throughout the study. Body weights and food consumption were recorded weekly for the first three months and monthly thereafter. Blood clinical chemistry was performed at weeks -1, 9, 14, 53 and 79. At week 79, the number of animals subjected to examination (10 per group) was supplemented by animals of the main groups to yield 10 samples per group. The following parameters were analysed: cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and sorbitol dehydrogenase.

From animals sacrificed at interim and terminal, the weights of brain, heart, liver, kidneys and testes (without epididymis) were recorded and a range of tissues were taken for microscopial examination. Necropsy with tissue preservation was also performed on animals which died or had to be sacrificed in moribund condition during the study. Histopathological examination was only conducted to liver. No significant clinical signs, behavioural changes or effects on survival were observed. Mortality was high, more than 60%, in all groups during the study (Table 27).

Table 27: Mortality in the main groups in 18 month study in male mice

	Dietary concentration of propiconazole (ppm)			
	0	**100**	**500**	**850**
Decedents until terminal sacrifice/no. at the beginning of the study	34/50 (68%)	33/50 (66%)	30/50 (60%)	32/50 (64%)

Body weights and cumulative body weight gains were equal to or significantly increased in 500 ppm and 850 ppm groups during the first 4 to 5 weeks of treatment. In the 850 ppm group, mean body

weight was significantly reduced at weeks 18–50, and mean cumulative body weight gain was significantly reduced at weeks 18-30 and 42-50 (Table 28). During weeks 14-50 the body weight gains in 850 ppm group were 11-19% lower, and in the 500 ppm group up to 10% lower than controls. Beyond the first year, no significant changes in mean body weights or body weight gains were observed.

Table 28: Cumulative mean body weight gains (g) in the 18 month study in male mice (selected weeks)

Week	Dietary concentration of propiconazole (ppm)			
	0	**100**	**500**	**850**
13	8.32	7.98 (4.1%↓)	7.75 (6.9%↓)	7.90 (5%↓)
26	10.55	10.47 (0.8%↓)	9.69 (8.2%↓)	9.17 (13.1%↓)
38	12.07	11.66 (3.4%↓))	11.01 (8.8%↓)	10.32 (14.5%↓)
50	12.32	12.20 (1.0%↓)	11.47 (6.9%↓)	10.65 (13.6%↓)

Treatment-related decreases in plasma cholesterol levels were recorded throughout the study in animals treated with 500 and 850 ppm; the decreases at weeks 9 and 14 were statistically significant in the 850 ppm group. Sorbitol dehydrogenase activities were increased in 850 ppm group at weeks 9 and 14 (increase at week 14 statistically significant).

The absolute and relative liver weights were significantly increased in 500 and 850 ppm groups, compared to controls at sacrifices on weeks 9, 53 and 79 (Table 29). Weights of other organs (brain, heart, kidney and testes were not affected by treatment.

Table 29: Mean liver weights in 18 month study in male mice

		Dietary concentration of propiconazole (ppm)			
		0	**100**	**500**	**850**
Interim Week 9	**Absolute (g)**	2.25	2.12	2.50 (11%↑)	**2.98*** (32%↑)
	Relative (/body weight)	59.28	59.38	65.00 (10%↑)	**78.68*** (33%↑)
Interim Week 53	**Absolute (g)**	2.58	2.55	2.93 (14%↑)	**2.86*** (11%↑)
	Relative (/body weight)	56.45	57.25	**63.87*** (↑13%)	**72.49*** (29%↑)
Terminal Week 79	**Absolute (g)**	2.58	2.58	2.93 (14%↑)	**3.07*** (19%↑)
	Relative (/body weight)	59.03	59.94	64.81 (10%↑)	**71.10*** (20%↑)

* $p<0.01$

The incidences of commonly occurring macroscopical findings (in the liver) at terminal sacrifice are summarised in Table 30. No dose-related increases in macroscopical findings were observed at week 9 and week 53 sacrifices. Enlarged livers were observed in higher numbers in the 850 ppm group than in other groups which confirms the increased liver weights recorded at necropsy. Increased numbers of animals having single masses in the liver were observed in all treated groups, and several nodules were found in the liver of one 850 ppm mouse.

Table 30: Incidences of macroscopial liver findings in 18 month study in male mice

Organ/Observation	**Dietary concentration of propiconazole (ppm)**			
	0	**100**	**500**	**850**
Terminal sacrifice, no. animals examined[a]	60	60	60	60
Enlarged	12	9	12	21
Mass	1	3	3	4
Nodule	0	0	0	1
Total masses and nodules	1	3	3	5

[a] Animals designated for oncogenicity evaluation (main group, 50) and clinical chemistry (10) including decedents.

Incidences of microscopial findings are summarised in Table 31. At interim sacrifices incidence of hepatocyte hypertrophy was significantly increased at 500 and 850 ppm groups. Moreover, incidence of fatty change (hepatocyte vacuolation) was significantly increased in 850 ppm group after at 9 weeks sacrifice. At terminal sacrifice incidence of hepatocyte hypertrophy was significantly increased in 500 and 850 ppm groups. Moreover, significantly increased incidences of pigmentation in Kupffer cells and foci of cellular change were observed in 850 ppm group. Foci of cellular change was further described as single eosinophilic foci of minimal to moderate in size. Hepatocyte necrosis (both monocellular and focal/multifocal) was slightly, but not significantly increased in 850 ppm group after 9 weeks and in terminal sacrifice. In the 850 ppm group incidence of hepatocellular adenoma (20% incidence compared to 2% in controls) and the combined incidence of hepatocellular adenoma and carcinoma (24% vs. 4% in controls) were significantly increased.

Table 31: Incidences of microscopial findings in 18 month study in male mice

Finding(number of animals affected)	**Dietary concentration of propiconazole (ppm)**			
	0	**100**	**500**	**850**
Sacrifice at week 9, no. of animals examined [a]	10	10	10	10
Fatty change (hepatocyte vacuolation)	0	2	2	**9*** **
Hepatocyte hypertrophy	0	0	**6***	**10*** **
Hepatocyte necrosis (focal/multifocal)	2	3	3	5
Hepatocyte necrosis (monocellular)	0	0	1	3
Lymphohistiocytic infiltration	2	2	1	6
Sacrifice at week 53, no. of animals examined [a]	10	10	10	10
Hepatocyte hypertrophy	1	3	6	**8****
Fatty change (hepatocyte vacuolation)	6	7	6	6
Lymphohistiocytic infiltration	5	8	5	7
Hepatocyte necrosis (focal/multifocal)	5	3	6	6
Hepatocyte necrosis (monocellular)	0	0	0	0
Hyperplasia, Kupffer cells	0	1	1	2
Foci of cellular change (eosinophilic)	0	0	2	1
Amyloidosis	5	5	8	9
Terminal sacrifice, no. of animals examined [a]	50	50	50	50
Hepatocyte hypertrophy	15	18	**28***	**29****
Kupffer cell pigmentation	3	5	3	**11***
Foci of cellular change (eosinophilic)	0	0	1	**6***
Fatty change (hepatocyte vacuolation)	21	14	15	13
Inflammatory cell infiltration	4	2	2	6
Lymphohistiocytic infiltration	20	23	20	20
Hepatocyte necrosis (monocellular)	9	14	7	13
Hepatocyte necrosis (focal/multifocal)	6	7	8	8
Amyloidosis	34	36	33	39
Hemangioma	0	0	1	1
Malignant lymphoma, systemic infiltration	0	1	1	1
Leukemia (myeloic), systemic infiltration	0	1	0	0

Hepatocellular adenoma	1 (2%)	0	3	**10**** (20%)
Hepatocellular carcinoma	1	3	2	2
Total hepatocellular tumours	2 (4%)	3	5	**12**** (24%)

[a] includes decedents *p<0.05, **p<0.01. Pairwise comparison to control group by Fisher exact test (non-neoplastic microscopy changes). Significant positive linear trend and pairwise comparison to control by method of Peto (1980) (neoplastic microscopy changes).

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A contemporary study was conducted in the same laboratory to collect reference control data of in-life parameters and post-mortem findings, including the incidence of liver tumours from CD-1 mice over a period of 18 months under standard laboratory conditions (DAR IIA, 5.5/05). Five groups per sex with 80 mice per group were used. After 3 months, an interim sacrifice of 10 mice per group/sex was performed. At the end of the test period, all surviving animals were necropsied and tissues collected. Inter-current deaths and moribund sacrificed animals were also necropsied with consecutive tissue preservation. 50 mice per group/sex were used for the evaluation of carcinogenic potential and were processed for histopathological examination. Only the incidences of primary neoplasia and of foci of cellular alteration in the liver of untreated male and female control CD-1 mice after 18 months in study were reported. In this study, **14-30%** males had hepatocellular neoplasia (Table 32). Incidence of hepatocellular adenoma ranged **6-18%**, and incidence of hepatocellular adenocarcinoma **8-16%** in five groups. In males, incidence of haemangioma was 2%, haemangiosarcoma 2%, and of foci of hepatic cellular alteration 2-4% per control group. No primary neoplasia or foci of cellular alteration were seen in the livers of females (zero incidence).

Table 32: Incidences of liver tumours in 18 month reference study in mouse

Finding	**Control Groups**				
	1	**2**	**3**	**4**	**5**
Number examined	50	50	50	50	50
Hepatocellular adenoma	9 (18%)	7 (14%)	3 (6%)	4 (8%)	7 (14%)
Hepatocellular adenocarcinoma	6 (12%)	8 (16%)	5 (10%)	4 (8%)	7 (14%)
Combined - Hepatocellular adenoma + adenocarcinoma	15 (30%)	12 (24%)	7 (14%)	7 (14%)	13 (26%)

The notifier also reported historical control data on CD-1 males from other laboratory: a historical control range of **6.0–18.4%** for hepatocellular adenomas and **0-12%** for hepatocellular carcinomas (four studies; 199 control animals, Charles River Lab.) and a mean adenoma incidence of **10.8%** (12 studies with a total of 770 controls, Charles River Lab. 1995).

In conclusion, propiconazole treatment resulted in lower body weights, lower body weight gain of up to 10% at 500 ppm and up to 19% at 850 ppm (up until week 52), reduced plasma cholesterol levels at 500 and 850 ppm and increased sorbitol dehydrogenase activities at 850 ppm. Enlarged livers and increased incidences of masses and nodules in 850 ppm group were observed at terminal sacrifice and this was confirmed by increased liver weights in 500 and 850 ppm groups. Histological signs of hepatotoxicity included hepatocyte hypertrophy at 500 and 850 ppm and increased incidence of foci of cellular change and Kupffer cell pigmentation at 850 ppm. Incidence of hepatocellular adenomas was significantly increased (20% vs. 2 % in controls) at terminal sacrifice in the 850 ppm group. The incidence of adenomas was slightly above the contemporary historical control incidence range (6-

18%) from the same laboratory, whereas the combined incidence of adenomas and carcinomas (24%) was within the contemporary historical control range (14-30%). The NOAEL after 18 months of treatment was 100 ppm, equivalent to an average daily intake of 11 mg/kg bw/day. The NOAEL for neoplastic effects (hepatocellular adenoma) was 500 ppm, corresponding to 59 mg/kg bw/day.

4.9.1.2 Carcinogenicity: inhalation.

No data are available

4.9.1.3 Carcinogenicity: dermal

No data are available.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

Additional studies to investigate the mode of action (MoA) for the propiconazole-induced liver tumours in mice have been conducted and are briefly described here. The first four studies were reported in DAR (1998) and Addendum 1 to the DAR (2002). Further mechanistic studies and the human relevance document were evaluated in 2015 for dRAR and for this CLH report.

Study 1. The effect of propiconazole on drug metabolizing enzymes in the livers of rats and mice (DAR II 5.8.6/02)

Male Tif:RAIf rats and Tif:MAGf mice were treated with 0, 20, 80, 160 or 320 mg/kg bw/day by gastric intubation during 14 consecutive days. Groups of 6 mice and 6 rats were used per dose. Mortality and clinical signs were checked daily. The animals were sacrificed after the termination of the treatment period, and the livers were removed and weighed. The microsomal and cytosolic fractions of the livers were prepared by centrifugation, the protein content was determined from the liver homogenates of all animals and the content of DNA from controls and animals of the highest dose. Concentrations of the following xenobiotic enzymes were determined in microsomal fractions: Cytochrome P-450, activity of ethoxycoumarin O-deethylase in the presence or in the absence of monooxygenase inhibitors, epoxide hydrolase, UDP-glucuronyltransferase. Glutathione S-transferase with 1-chloro-2,4-dinitrobenzene as substrate was determined in cytosolic fractions, and the activity of glutamyltranspeptidase in rat liver supernatants. Livers from 2 rats and 2 mice of control groups and 2 rats and 3 mice from the highest dose group were prepared and examined by electron microscopy. There were no positive controls (e.g. treated with phenobarbital or 3-methylcholanthrene).

Results: Propiconazole had no effect on body weight gain of either species at the tested doses. A dose-dependent increase in relative liver weights was observed at all the dose levels of propiconazole in both species. Electron microscopy of histological slides revealed marked proliferation of the smooth endoplasmic reticulum membranes of hepatocytes in both species at the daily dose of 320 mg/kg bw. Statistically significant increases in practically all xenobiotic enzymes were noted in both species, usually at 80 mg/kg bw/day and higher concentrations, with a somewhat more pronounced effect in rat. Especially the enzymes known to be structurally associated with the endoplasmic reticulum were increased by propiconazole treatment. For example, at the highest dose, the increase of cytochrome P-450 content was 3.3-fold in rats and 4.0-fold in mice. The activity of microsomal

epoxide hydrolase was clearly lower in mice than in rats, whereas activities of UDP-glucuronosyltransferase and glutathione S-transferase were significantly higher.

Conclusions: Propiconazole caused dose-dependent increase of liver weights in both rats and mice, with clearly discernible changes in the ultrastructural organisation of hepatocytes. Propiconazole was an efficient inducer of xenobiotic metabolism in both rat and mouse. The profiles of liver enzyme induction in rat and mouse by propiconazole were found to be different in some respects, especially concerning activities of cytochrome P-450 enzymes, epoxide hydrolase, and glutathione S-transferase. The possible explanation to the observed differences in hepatic tumour formation between the two species and the sex differences noted in mice, could be based on the observed differences in xenobiotic metabolism.

Study 2. Tumor promotion study with propiconazole in rat (DAR II 5.8.6/01)

Male and female Tif:RAIf rats pretreated with 15 mg/kg N-nitrodiethylamine (i.p.) or vehicle were used for testing the tumor promoting activity of propiconazole administered in feed. The vehicle control and N-nitrosodiethylamine (DENA) treatment groups were further divided into three dietary treatment groups receiving: 1) control diet, 2) 500 ppm phenobarbital (positive control), or 3) 2000 ppm propiconazole. The duration of the study was 56 days, with interim sacrifices after 14 and 28 days. Five males and five females were used per group and sacrifice time. Liver weights were recorded and sections of liver were examined for histopathological changes by staining with hematoxyline and eosine (HE) and periodic acid Schiff (PAS). Hepatocyte structure in histopathological sections was studied by detecting cellular ɣ-glutamyltransferase (ɣ-GT) activity. The ɣ-GT stained sections were used to determine the presence and histological nature of focal and diffuse ɣ-GT-positive changes. Statistical tests were not performed due to the small number of animals in the groups. Differences greater than 20% were regarded as possible treatment-related effects.

Results: No mortalities occurred during the treatment period, and no changes were observed in body weight development or in food intake. Treatment with propiconazole and phenobarbitone resulted in clearly increased liver weights, in comparison with controls, after 28 days of treatment up to the termination of the study. These effects were somewhat more pronounced in males. Propiconazole or phenobarbital alone enhanced the formation of ɣ-GT positive foci smaller than 0.01 mm^2 by increasing their number but not their size. When propiconazole or phenobarbital were given after pretreatment with DENA, more and larger foci were observed. More foci smaller than 0.01 mm^2 were found after 56 days of treatment with 2000 ppm propiconazole than with 500 ppm phenobarbital in DENA pretreated rats. Similar numbers of foci larger than 0.01 mm^2 were found after treatment with propiconazole and phenobarbital. In vehicle control + control diet animals, female rats exhibited much higher numbers of ɣ -GT positive foci than males, where only one focus was observed at 28 days.

Conclusions: Propiconazole acts as a promoter of proliferative changes in rat liver at dietary concentrations of 2000 ppm. The tumour promoting efficiency of propiconazole is similar to or higher than the one exhibited by phenobarbital.

Study 3. Assessment of hepatic cell proliferation in male mice (DAR IIA/5.8)

The study was designed to characterize the extent and time dependence of hepatocyte proliferation at tumorigenic doses of propiconazole (PCZ). Eight groups of five, young adult male CD-1 mice were fed diet containing 0 (control), 850 or 2500 ppm propiconazole (equivalent to 0, 127, 353 or 139 mg/kg bw/day) or 850 ppm phenobarbital (PB, 139 mg/kg bw). One group per treatment was killed after 1, 2, 3, 4, 7, 14, 28 and 60 days. To examine hepatocyte cell proliferation, each animal received a single i.p. injection of BUdR (100 mg/kg bw) 2 h before sacrifice. Cell proliferation was assessed by BUdR-immunohistochemistry/image analysis.

Results: No unscheduled mortalities occurred during the study and no treatment related clinical signs were reported with either PCZ or PB. Body weight development and food consumption was suppressed during the first days of treatment at the 2500 ppm dose level. Carcass weights were significantly reduced at days 2, 3 and 4 in high dose animals. No effects on bodyweight development, food consumption or carcass weights were noted in 850 ppm PCZ or PB groups. Rapid time- and dose-related increases in absolute and relative liver weights were observed in PCZ treated animals. The maximum increase in absolute liver weight was 147% in the low dose PCZ group and 241% in the high dose group, compared to controls. The duration of the weight gain period was 3 days for the low dose and 14 days for the high dose. Treatment with PB resulted in a liver weight maximum of 175%, compared to the control, and the weight gain reached a plateau within 4 days.

Enlarged and/or speckled livers were found at macroscopic examination in animals treated with PCZ or PB. One hepatic nodule was found in each of the high and low dose PCZ groups. The macroscopic changes were correlated with hepatocellular hypertrophy observed at microscopical examination.

Hepatocellular hypertrophy was found in all low and high PCZ dose animals immediately after treatment. At 850 ppm PCZ the mean severity, increasing with time, ranged from minimal (1-day treatment) to moderate/marked (28- and 60-day treatment). At 2500 ppm the mean severity of hypertrophy ranged from minimal (day 1) to marked (days 28 and 60). Treatment with 850 ppm PB led to a progression of hepatocellular hypertrophy similar to the one observed in the 2500 ppm PCZ group. The hypertrophy caused by PCZ treatment affected all lobular compartments of the liver (centrilobular: strong, midzonal: mild, periportal: weak), whereas PB almost exclusively affected centrilobular or midzonal hepatocytes. The centrilobular-periportal gradient of hypertrophy seemed to be weak in PCZ groups and strong in the PB groups.

Hepatocellular necrosis (minimal) was observed in several animals of the 850 ppm PCZ group, especially after prolonged treatment. Minimal/moderate necrosis of the liver was found in almost all 2500 ppm PCZ and 850 ppm PB treated animals. The necroses were usually found in centrilobular/midzonal areas, typically affecting hypertrophic hepatocytes.

Mitotic activity of hepatocytes was increased in most treated animals during days 2 – 4 of exposure. The maximum incidence and severity was observed on day 2 for both PCZ dose levels (moderate/marked; all animals) and for PB (marked; all animals). The increased mitotic activities seemed to become less pronounced with time; treatment related differences were no longer observed by week 8. Mitotic hepatocytes were mainly found at the centrilobular/midzonal location.

Cytoplasmic vacuolation was divided into panlobular cytoplasmic vacuolation and centrilobular cytoplasmic vacuolation. Panlobular vacuolation (minimal) was observed in 1–3 animals per group during days 3–7 in the PCZ groups, and in 2 animals of the day 3 PB group. Centrilobular vacuolation was observed towards the end of the treatment, and with a much higher incidence and severity (practically all animals from day 7 to 60; minimal to marked) in 2500 ppm PCZ treated animals than in 850 ppm PCZ (days 4 [1 mice] and 60 [3 mice]; minimal) or PB (day 28 [1 mice]; minimal) treated animals.

Hepatocyte proliferation. The 850 ppm PCZ groups exhibited significantly increased mean BUdR labeling indices on days 1 – 4. Peak values of relative mean labeling indices (maximum 3662% of control) were found on days 1 and 2; the values returned to control level on day 7. The 2500 ppm PCZ group showed significantly increased mean labelling indices on days 1–7. The peak relative mean labelling index value (4862% of control) was noted on day 2. From day 14 on the values dropped to values slightly above control levels. Treatment with 850 ppm PB resulted in statistically increased mean labelling indices on days 1–7. A peak relative mean labelling index value (9517% of control) was found on day 2. From day 14 on the values dropped to values slightly above control levels. BUdR-labeled hepatocyte nuclei were mainly found in centrilobular/midzonal regions in groups treated with PCZ or PB for 4–7 days. They appeared occasionally to be arranged in a ring-like fashion in the midzonal area.

Conclusions: Treatment with propiconazole at 850 and 2500 ppm for up to 60 days caused a prominent, time- and dose-related hepatomegaly. The liver enlargement was caused by a sharp and transient induction of hepatocellular proliferation and to a time- and dose-related increase in the severity of hepatocellular hypertrophy. The centrilobular-periportal gradient of hypertrophy seemed to be weak in PCZ groups and strong in the PB groups. Since the most pronounced induction of hepatocellular proliferation was observed with phenobarbital together with intermediate liver weight increase, it seems that hyperplasia plays a more important role than hypertrophy for phenobarbital induced liver growth, whereas for propiconazole it seems to be the other way round. Hepatocellular cytoplasmic vacuolation was noted at 2500 ppm, mostly occurring at later time-points. Hepatocellular necrosis was also increased in a time- and dose-dependent manner. In general, the temporal pattern of propiconazole induced hepatocyte proliferation was the same as for phenobarbital, suggesting that propiconazole is a phenobarbital-like mitogen in the male mouse liver.

Study 4. Effects on biochemical parameters in the liver following administration to male mice (DAR IIA/5.8)

Liver enzyme induction was investigated in male CD-1 mice after administration of tumorigenic doses of propiconazole. Groups of 6 young adult male CD-1 mice were treated for 14 days with propiconazole (PCZ: 92.4% purity) at dietary doses of 0, 850 and 2500 ppm, and with the reference compound phenobarbital (PB) at 850 ppm, corresponding to mean daily doses of 0, 149, 578 and 145 mg/kg bw/day, respectively.

Results: No clinical signs of toxicity were noted. PCZ had no effect on carcass weight, whereas PB increased the carcass weight slightly to 109% of the control. Both absolute and relative liver weights were increased by both doses of PCZ and PB to 150 – 200% of the control.

The major treatment-related biochemical alterations in the liver at treatment with PCZ at 2500 ppm comprised of increases in total microsomal cytochrome P450 content, cytochrome P450 isoenzyme Cyp2b-10-dependent O-depentylation of 7-pentoxyresorufin, Cyp2a-dependent coumarin 7-hydroxylase, testosterone oxidation at several positions, content of barbiturate- and steroid-inducible microsomal cytochrome P450 isoenzymes of subfamilies Cyp2b and Cyp3a. These findings indicate a PB type of induction of xenobiotic metabolising enzymes of the liver.

Table 33: Biochemical parameters in the liver of male mice

Biochemical / immunochemical parameter	Relative amount or activity (% of control)		
	Phenobarbital	Propiconazole	
	850 ppm	850 ppm	2500 ppm
Strong to moderate effects with propiconazole			
Microsomal cytochrome P450 content	239	300	389
Microsomal pentoxyresorufin O-depentylase (PROD)	3534	3024	5524
Microsomal coumarin 7-hydroxylase	480	534	2384
Microsomal testosterone 2β-hydroxylation	466	298	531
Microsomal testosterone 6α-hydroxylation	779	502	715
Microsomal testosterone 6β-hydroxylation	500	366	524
Microsomal testosterone 15β-hydroxylation	526	316	977
Microsomal testosterone 16β-hydroxylation	5.7 [1)]	5.2 [1)]	6.4 [1)]
Microsomal testosterone oxidation to androstenedione	179	589	652
Total microsomal testosterone oxidation	356	440	555
Microsomal epoxide hydrolase	179	172	321
Immunoblot Goat anti Rat CYP2B1 (Cyp2b) Band 1	2743	2608	3049
Immunoblot Goat anti Rat CYP2B1 (Cyp2b) Band 2	581	810	579
Immunoblot Goat anti Rat CYP2B1 (Cyp2b) Band 3	351	439	617
Immunoblot MAb p6 (Cyp3a)	577	658	1068
Slight effects with propiconazole			
Microsomal ethoxyresorufin O-deethylase (EROD)	232	219	388
Microsomal testosterone 2α-hydroxylation	0 [1)]	2.7 [1)]	2.7 [1)]
Microsomal testosterone 16α-hydroxylation	194	214	262
Microsomal lauric acid 11-hydroxylase	271	267	305
Microsomal lauric acid 12-hydroxylase	163	153	161
Microsomal UDP-glucuronosyltransferase (UDPGT)	156	156	139
Cytosolic glutathione S-transferase (GST)	187	158	184
No effects with propiconazole			
Microsomal testosterone 7α-hydroxylation	229	175	125
Microsomal protein content	97	109	113
Cytosolic protein content	92	97	94
Immunoblot MAb d15 (Cyp1a)	86	124	89
Immunoblot MAb clo4 (Cyp4a)	129	140	157

[1)]Absolute values, control value below limit of detection

Treatment with PCZ at 850 ppm generally resulted in a lower but still clear induction for all biochemical parameters, which demonstrates a dose-dependency for the liver enzymatic induction.

The lack of an effect of PCZ on the content of microsomal cytochrome P450 isoenzymes of subfamilies Cyp1a and Cyp4a as well as only minor effects on microsomal cytochrome P450 isoenzyme Cyp1a-1-dependent O-deethylation of 7-ethoxyresorufin and Cyp4a-dependent 12-hydroxylase of lauric acid exclude the mode of action as a 3-methylcholanthrene- or peroxisome proliferator-type inducer.

Treatment with the reference compound PB at a dose of 850 ppm resulted in a similar induction profile as with PCZ. Alterations included total microsomal cytochrome P450 content, O-dependentylation of 7-pentoxyresorufin, microsomal coumarin 7-hydroxylase activity, testosterone oxidation, content of microsomal cytochrome P450 isoenzymes of subfamilies Cyp2b and Cyp3a. The induction by PB was quantitatively similar to that seen with PCZ at 850 ppm. Differences to PCZ were a lower extent of induction of androstenedione formation from testosterone and the lack of induction of testosterone 2α-hydroxylation.

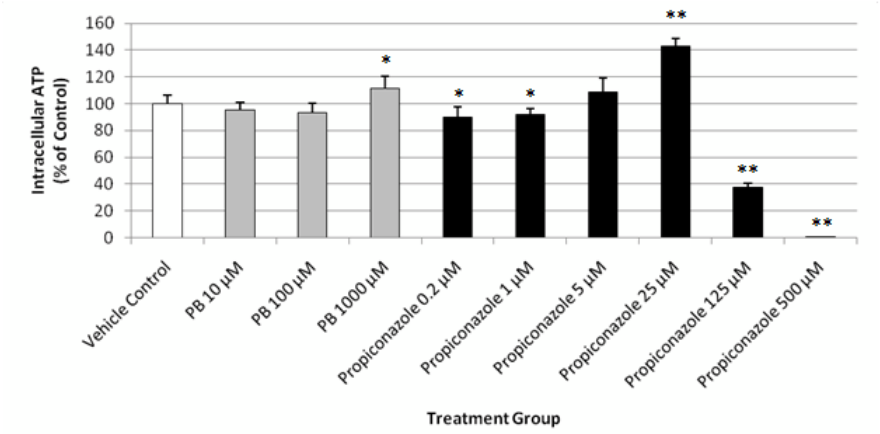
Conclusions: Subchronic treatment of male mice with 850 and 2500 ppm propiconazole or 850 ppm phenobarbital caused strong and qualitatively similar induction effects on liver weight and biochemical liver parameters. Quantitatively comparable effects were observed with 850 ppm propiconazole and phenobarbital, whereas more pronounced effects were observed with 2500 ppm propiconazole. Thus propiconazole was found to be a strong phenobarbital-type inducer of xenobiotic metabolising enzymes in the mouse liver. The lack of an effect on the content of microsomal cytochrome P450 isoenzymes of subfamilies Cyp1a and Cyp4a as well as only minor effects on microsomal cytochrome P450 isoenzyme Cyp1a-1-dependent O-deethylation of 7-ethoxyresorufin and Cyp4a-dependent 12-hydroxylase of lauric acid exclude the mode of action as a 3-methylcholanthrene- or peroxisome proliferator-type inducer.

<u>Study 5.</u> Cytochrome P450 2b, 3a and DNA-synthesis induction in cultured male mouse hepatocytes (dRAR B6.8.2.2)

This study investigated the ability of propiconazole to induce cytochrome P450 2b10 (Cyp2b10) and Cyp3a11 expression and changes in cell proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) in primary male CD-1 mouse (out-bred Hsd:ICR (CD-1)) hepatocyte cultures. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) were included as positive controls for induction of Cyp isoforms and cell proliferation, respectively. Hepatocytes were exposed to propiconazole at 6 concentrations (0.2, 1, 5, 25, 125 and 500 µM), to PB at 3 concentrations (10, 100 and 1000 µM) or vehicle (0.5 % v/v dimethyl sulfoxide [DMSO]) alone for 96 hours. Cytotoxicity was measured as the change in cellular adenosine-5'-triphosphate (ATP) released from viable hepatocytes, low amounts relative to vehicle controls indicating low viability or increased cytotoxicity. The number of hepatocytes undergoing replicative DNA synthesis (S-phase of the cell cycle) was determined immunocytochemically following the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into hepatocyte nuclei over the last 72 hours of culture. Levels of Cyp2b10 and Cyp3a11 mRNA were assessed by quantitative RT-PCR using target gene specific primers and probes. The expression of Cyp2b10 and Cyp3a11 protein for selected concentrations of propiconazole in cultured mouse hepatocytes was assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

Results: *Cytotoxicity*. Treatment with 125 and 500 µM propiconazole resulted in significant cytotoxicity with reductions of intracellular ATP levels to 37.3 % and 0.8 % of control, respectively. Thus, replicative DNA synthesis as well as Cyp2b10 and Cyp3a11 mRNA data could either not be measured at these concentrations or were consequently excluded from data analysis. Treatment with 0.2 µM and 1 µM propiconazole resulted significant (90 % and 92.2 %of control, respectively), but less pronounced reductions in intracellular ATP. The tested PB concentrations (up to 1000 µM) did not induce cytotoxicity.

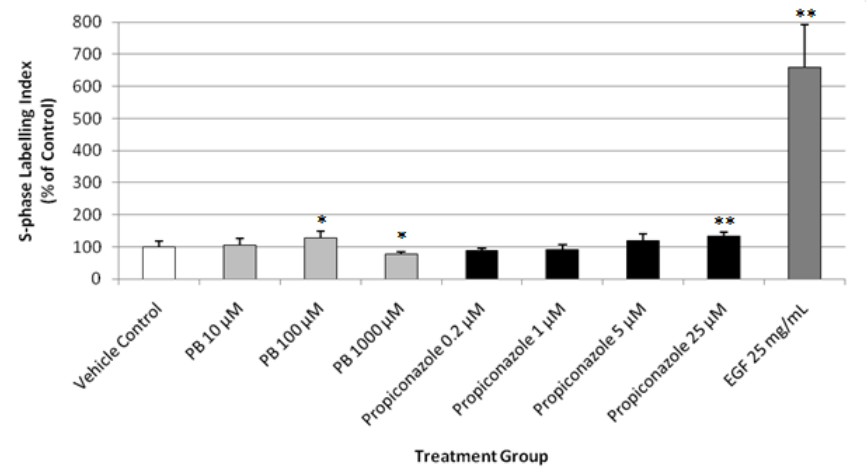
Figure 2. Intracellular ATP in mouse hepatocytes.



Values are Mean ± SD (n = 6). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$; **$p<0.01$.

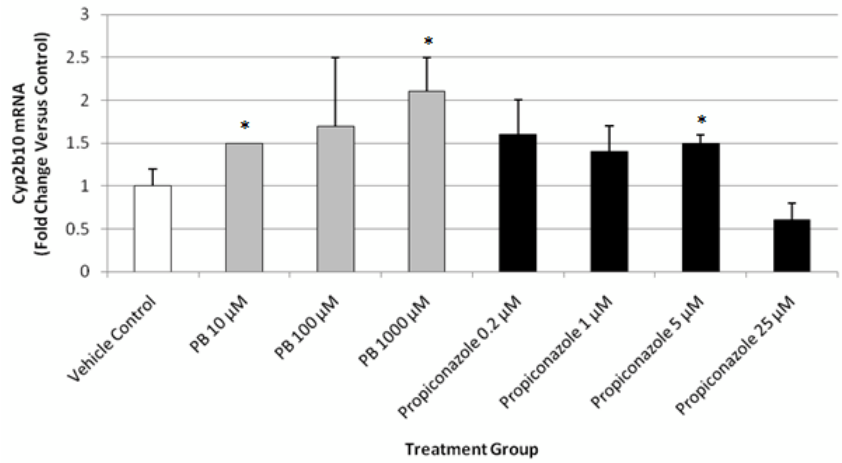
Proliferation and Cyp expression. Treatment with 5 µM propiconazole resulted significant increase in expression of Cyp2b10 mRNA (but not protein) and slight increase in Cyp3a11 expression. Treatment with 25 µM propiconazole resulted in statistically significant increases in replicative DNA synthesis, intracellular ATP levels and expression of Cyp3a11 mRNA and protein. A slight decrease in Cyp2b10 expression was observed at this concentration of propiconazole. Treatment with 100 µM PB resulted significant increase in replicative DNA synthesis and 1000 µM PB resulted increased expression of Cyp2b10 and Cyp3a11 and significant increase in intracellular ATP.

Figure 3. Replicative DNA synthesis (S-phase)



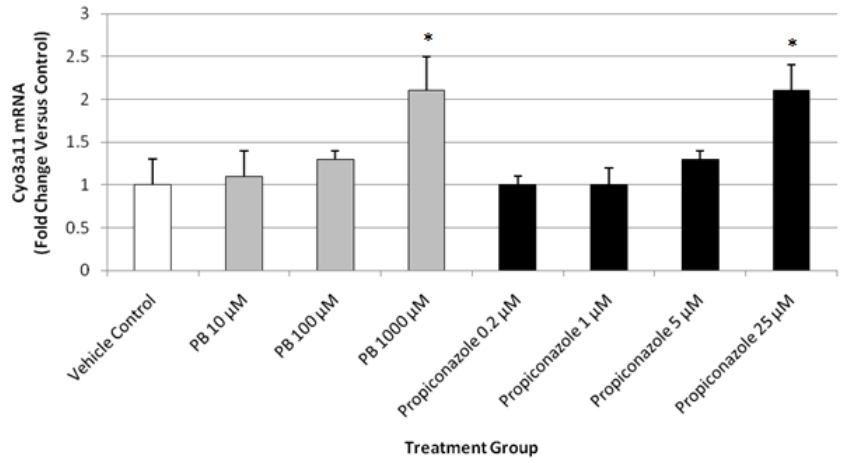
Values are Mean ± SD (n = 5). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$; **$p<0.01$.

Figure 4. Cyp2b10 mRNA expression



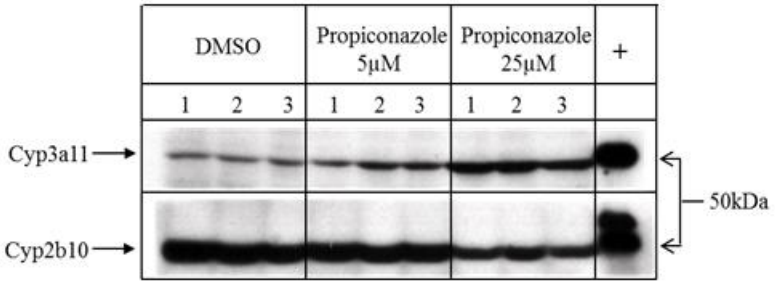
Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$.

Figure 5. Cyp3a11 mRNA expression



Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$.

Figure 6. Cyp3a11 and Cyp2b10 protein expression



Mouse hepatocyte proteins (40µg) were resolved on 7.5% polyacrylamide gels and transferred to PVDF membranes using standard methods. Membranes were probed with either rabbit anti-CYP3A (CH32, diluted 1/4000) or rabbit anti-CYP2B (CH4, diluted 1/4000); the secondary antibody was HRP-linked donkey anti-rabbit IgG (GE Healthcare, diluted 1/4000). Recombinant mouseCyp3a11 and Cyp2b10 were used as positive controls for Cyp3a11 and Cyp2b10, respectively.

Conclusions: Based on reduction in intracellular ATP, 125 µM and 500 µM propiconazole is considered to be cytotoxic to mouse hepatocytes. Significantly reduced ATP levels at 0.2 µM and 1 µM suggest that propiconazole could be cytotoxic also at lower concentrations. EGF induced cell proliferation, whereas PB induced Cyp2b10, Cyp3a11 and cell proliferation consistent with activation of the constitutive androstane receptor (CAR). This demonstrates sensitivity of the assay in mouse hepatocytes. 5 µM and 25 µM propiconazole resulted in the induction of Cyp2b10, Cyp3a11 and cell proliferation consistent with activation of CAR.

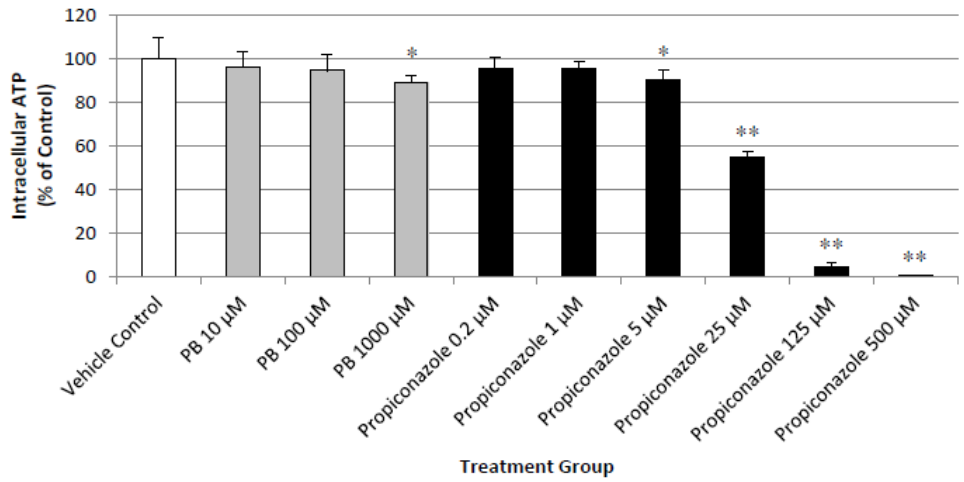
<u>Study 6</u>. Cytochrome P450 2B, 3A and DNA- synthesis induction in cultured male human hepatocytes (dRAR, B.6.8.2.3)

This study investigated the ability of propiconazole to induce cytochrome P450 (CYP) 2B6 (CYP2B6), CYP3A4 and cell proliferation (change in replicative DNA synthesis [S-phase of the cell cycle]) in isolated male human hepatocyte cultures. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) were included as positive controls for induction of CYP isoforms and cell proliferation, respectively.

Hepatocytes were exposed to propiconazole at 6 concentrations (0.2, 1, 5, 25, 125 and 500 µM), PB at 3 concentrations (10, 100 and 1000 µM) or vehicle (0.5% v/v dimethyl sulfoxide [DMSO]) alone for 96 hours. Cytotoxicity was measured as the change in cellular adenosine-5'-triphosphate (ATP) released from viable hepatocytes. The number of hepatocytes undergoing replicative DNA synthesis (S-phase of the cell cycle) was determined immunocytochemically following the incorporation of 5-bromo-2'-deoxyuridine (BrdU, 10 µM) into hepatocyte nuclei over the last 72 hours of culture. Levels of human CYP2B6 and CYP3A4 mRNA were assessed by quantitative RT-PCR using target gene specific primers and probes. The expression of CYP2B6 and CYP3A4 protein for selected concentrations of propiconazole in cultured human hepatocytes was assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

Results: Treatment with 5, 25, 125 and 500 µM propiconazole resulted in statistically significant lower intracellular ATP, with levels being reduced to 90, 55, 4 and 0.4 % of control, respectively. The cytotoxicity observed following treatment with 125 and 500 µM was considered excessive, and therefore replicative DNA synthesis, CYP2B6 and CYP3A4 mRNA data could either not be measured at these concentrations or were excluded from data analysis. Treatment with 1000 µM PB reduced intracellular ATP levels to 89% of control values.

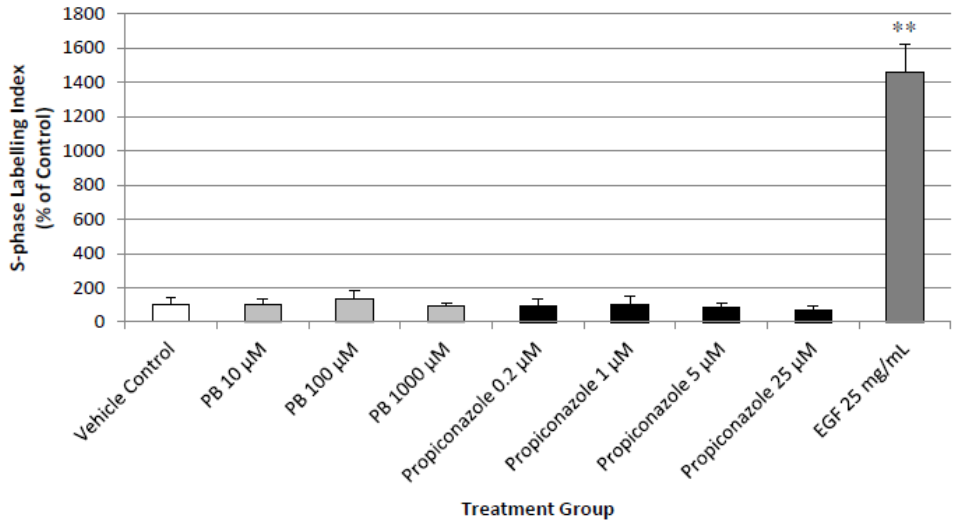
Figure 7. Intracellular ATP in human hepatocytes



Values are Mean ± SD (n = 6). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05;**p<0.01.

Treatment with EGF resulted in significant increase in replicative DNA synthesis to 14.6-fold control, indicating that the hepatocytes could proliferate following exposure to proliferative stimuli. Neither propiconazole nor PB induced increases in replicative DNA synthesis (S-phase of the cell cycle), indicating that neither compound induced cell proliferation.

Figure 8. Replicative DNA synthesis (S-phase)



Values are Mean ± SD (n = 5). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05;**p<0.01.

Treatments with 100µM and 1000 µM PB and 5 µM and 25 µM propiconazole resulted in statistically significant increases in both CYP2B6 and CYP3A4 mRNA. These increases indicate increased expression of the *cyp2b6* and *cyp3a4* genes. Hence, CYP3A4 mRNA induction was clearly lower at 25 µM compared to 5 µM propiconazole. This possibly reflects increasing cytotoxicity. CYP3A4 mRNA levels were significantly increased also after treatment with 10 µM PB and 1 µM propiconazole. Treatment with 5 and 25 µM propiconazole resulted in increased CYP3A4 protein

amount compared to the control group. CYP2B6 protein levels appeared unaffected in hepatocytes treated with 5 µM propiconazole and decreased in hepatocytes treated with 25 µM propiconazole (data not shown).

Figure 9. CYP2B6 mRNA expression

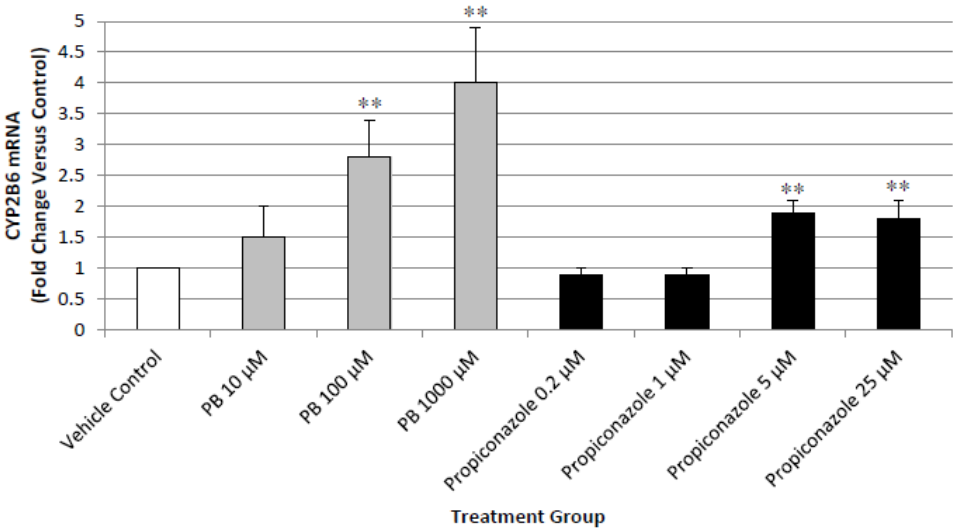
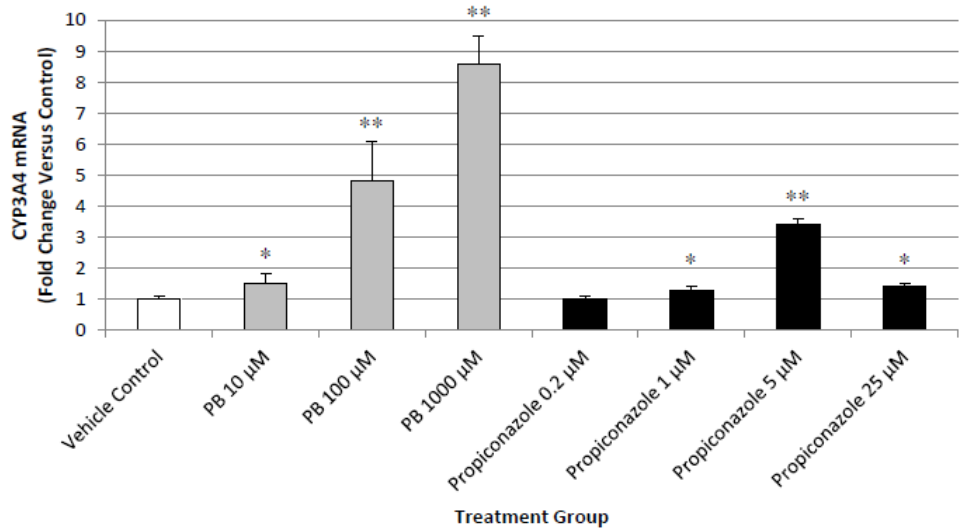


Figure 10. CYP3A4 mRNA expression



Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$;**$p<0.01$.

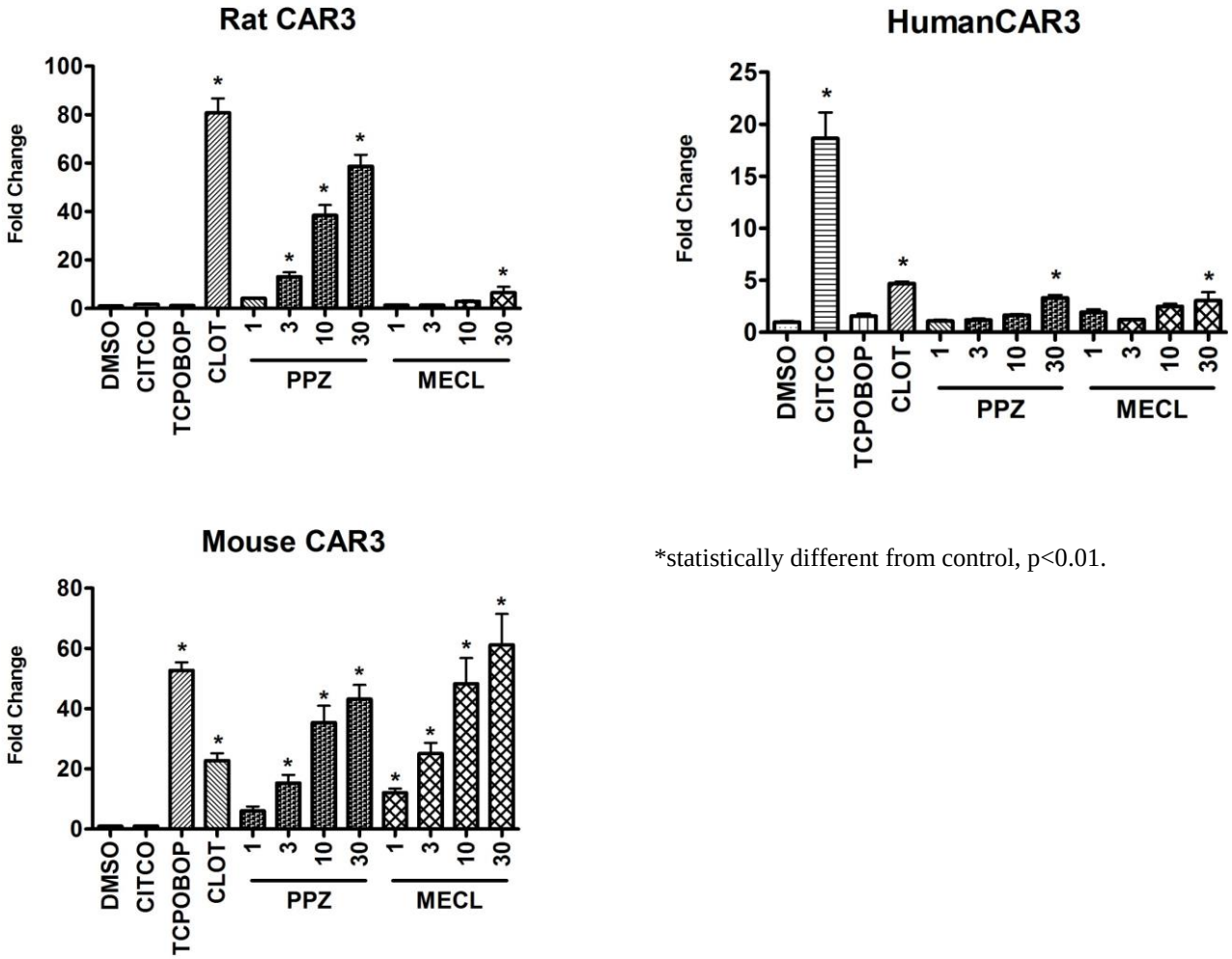
Conclusions: Propiconazole concentrations 25 µM, 125 µM and 500 µM were cytotoxic to cultured male human hepatocytes. PB induced CYP2B6 and CYP3A4 transcripts without affecting cell proliferation, while treatment with EGF resulted in increased cell proliferation. Treatment with propiconazole resulted in the induction of CYP2B6 and CYP3A4 without affecting cell proliferation. This is consistent with species differences in CAR and PXR receptors between humans and rodents.

Study 7. CAR3 direct activation assay with mouse, rat and human CAR (dRAR, B.6.8.2.4)

Propiconazole was tested for its ability to directly activate the constitutive androstane receptor (CAR, NR1I3) in a reporter assay. cDNA expression vectors for CAR3 variants of mouse, rat and human CAR were transfected into COS-1 cells, along with necessary cofactors and a CYP2B6 response element-luciferase reporter construct. After a suitable expression time, the cells were incubated with propiconazole at concentrations of 1, 3, 10, and 30 µM. The direct CAR activator meclizine was also incubated at these same concentrations, and model direct-acting substrates for mouse, rat or human CAR were each incubated at a single concentration. Light emission from the luciferase reporter was quantified to indicate the extent of CAR activation upon incubation with suspected ligands, including propiconazole. Results were reported as normalized luciferase activity and fold change compared to a DMSO solvent control. Propiconazole was tested in the human, mouse and rat assays, using the respective species CAR3 reporter constructs.

Results: A strong concentration-dependent activation of rodent CAR3 by propiconazole was observed, with up to 40-fold activation of mouse CAR3 and up to 60-fold activation of rat CAR3. In contrast, the human CAR3 response was only statistically significant at 30 µM – the highest dose tested, and this response only represented a 3-fold activation above solvent control.

Figure 11. A comparison of CAR activation by propiconazole and positive control compounds in rat, mouse and human CAR3 (fold change relative to DMSO control)



*statistically different from control, p<0.01.

The model activators CITCO (prototype human CAR ligand), TCPOBOP (prototype mouse CAR ligand) and clotrimazole (CLOT) produced robust responses in human, mouse and rat CAR3 constructs, respectively. Meclizine (MECL) was also tested and produced a concentration-dependent response that was much more marked with mouse CAR3 than with human or rat CAR3.

Conclusion: Results of this study indicate that propiconazole is a direct CAR activator in mouse, rat and human. Under the conditions of this assay, the activation of rat CAR with propiconazole was strongest, whereas responsiveness of human CAR to propiconazole was much weaker than mouse and rat CAR. This suggests quantitative difference between rodent CAR and human CAR with respect to their direct activation by propiconazole.

Document 8. Green RM, Peffer RC, Currie R. (2014). Propiconazole – Human Relevance Framework Assessment of Liver Tumor Induction in Mice: Assessment.

The document contains an overview of the notifier´s evaluation of the relevancy of propiconazole to human health in the context of mouse carcinogenicity. The report in its original form is provided as an attachment to section 13 of the CLH dossier. Notifier´s summary of the report is provided as Annex 8 of this CLH dossier.

Notifier´s executive summary: "This document assesses a postulated mode of action (MOA) for these propiconazole-induced liver tumors using the framework developed by the IPCS and ILSI/HESI. Finally, the human relevance of the identified MOA is assessed.
The available data for PPZ support a proposed MOA in male mice involving:

- Activation of the constitutive androstane receptor (CAR) nuclear receptor
- Increased expression of CAR-responsive pro-proliferative and anti-apoptotic genes
- Associative events that are mediated by CAR activation, including increased CYP enzymes (primarily CYP2b and CYP3a), hepatocellular hypertrophy, and increased liver weight.
- Transient increase in hepatocellular proliferation and decreased apoptosis
- Clonal expansion resulting in increased incidence of altered hepatic foci
- Eventual progression to form hepatocellular tumors.

The available data also demonstrates that this MoA is not relevant for human hazard/risk assessment purposes, due to qualitative differences in response to CAR activation between mice and humans. Experimental data demonstrate that PPZ does not produce the key event of cell proliferation in human liver cells *in vitro.* This pattern of effects matches the known species differences that have been demonstrated for other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MOA for PPZ between mice and humans. In summary, the data support the conclusion that PPZ does not pose a carcinogenic hazard to humans."

Overall discussion of mechanistic studies relating to mouse liver tumour induction

According to Elcombe *et al* (2014) the CAR-mediated pathway is associated with the following key events and associated events. Activation of CAR receptor (key event 1.) and altered expression of CAR-responsive genes (key event 2.) is followed by liver enzyme induction (CYP), increased liver weight, hepatocyte hypertrophy (associated events) and increased cell proliferation (key event 3.), which leads to clonal expansion leading to altered foci (key event 4) and formation of adenomas and carcinomas (key event 5.).

Propiconazole was shown to directly activate mouse, rat and human CAR in a reporter assay and to induce CAR-responsive genes Cyp2b10, Cyp3a11 and cell proliferation in mouse hepatocyte cultures. In human hepatocyte cultures, consistently with species differences in CAR and PXR receptors (reviewed in Elcombe et al. 2014), propiconazole treatment resulted in induction of CYP2B6 and CYP3A4 without affecting cell proliferation. Altered expression of CAR-responsive genes following propiconazole treatment has been demonstrated also in a number of other studies (e.g. Ward *et al* 2006, Goetz and Dix 2009, Nesnow *et al* 2011) and CAR activation has been shown to be involved in tumorigenic action of other conazoles (Tamura *et al.* 2015). Propiconazole caused dose-related increase in hepatocellular proliferation and in the severity of hepatocellular hypertrophy leading to pronounced hepatomegaly (DAR IIA/5.8). After prolonged *in vivo* treatment eosinophilic altered hepatic foci were observed (DAR IIA5.5/03, IIA5.5/05). Overall, there is clear evidence for involvement of CAR in propiconazole-induced tumourgenesis in the liver of CD mice.

Nevertheless, there are data suggesting that in addition to CAR activation, other mechanisms may be involved in propiconazole-induced tumourgenesis in mice liver. Although the general pattern of responses to propiconazole and phenobarbital, a known CAR activator, were similar in mice liver *in vivo* and hepatocytes *in vitro*, the studies also show differences. The centrilobular-periportal gradient of hypertrophy in the liver was weak with propiconazole and strong with phenobarbital. Moreover, hyperplasia seems to play a more important role than hypertrophy for phenobarbital induced liver growth, whereas for propiconazole it seems to be the other way round. In mice hepatocytes propiconazole-induced proliferation did not correlate with Cyp2b induction (highest rate of cell proliferation at 25 µM, but lowest level of Cyp2b mRNA) and Cyp2b protein amounts were not induced in the study (dRAR B 6.8.2.2).

Alternative MoAs for propiconazole-induced hepatocellular carcinogenesis are discussed in the "Human relevance framework assessment" submitted by the notifier (Green et al 2014). Propiconazole is not genotoxic and peroxisome proliferation and aromatic hydrocarbon receptor (AhR) P450 induction can be excluded, since propiconazole caused only slight or no increase in lauric acid 12-hydroxylase activity, Cyp4a protein levels, EROD activity and Cyp1a protein levels (DAR IIA/5.8). Estrogenic stimulation (negative ER binding and uterotrophic assays, no effects on estrogen-sensitive tissues) and altered cholesterol biosynthesis (altered cholesterol metabolism is likely consequence of CAR-activation) are also excluded. In addition, the notifier states that the cytotoxic mode of action can be excluded, since propiconazole induces transient early hepatocyte proliferation in contrast to classical hepatotoxic agents (e.g. chloroform and carbon tetrachloride) that induce sustained regenerative proliferation and development of long-term fibrotic changes. Propiconazole was cytotoxic to mouse and human hepatocytes *in vitro*, whereas no cytotoxicity was reported with phenobarbital (dRAR B 6.8.2.2, B.6.8.2.3). Moreover, degenerative lesions in the liver (vacuolation, necrosis, inflammation), prominent lobular architecture of the liver, and increases in serum ALT, AST and sorbitol dehydrogenase suggesting cytotoxic MoA were reported at tumorigenic propiconazole doses (DAR IIA 5.8, DAR IIA 5.5/03, DAR II 5.3.2/04, and repeated dose studies). However, these findings are not fully consistent with cytotoxic MoA: necrosis was only minimal to moderate in severity and primarily observed in short term studies, proliferative response was transient and there was no sustained regenerative growth.

Diverging views of tumorigenic mechanism of propiconazole have been presented based on studies published in open scientific literature (e.g Allen *et al*., 2006, Ward *et al.*, 2006, Nesnow *et al.*, 2009, Ortiz *et al.* 2010, Nesnow *et al* 2011, Hester *et al.* 2012). Transcriptomic analyses of two different laboratories indicated CAR-activation in propiconazole treated mice liver (Ward *et al.* 2006, Nesnow *et al*. 2009). Yet, based on differences in transcriptional profiles of phenobarbital and two conazoles, propiconazole and triadimefon, Nesnow *et al* (2009) concluded that the MoA´s of phenobarbital and conazoles are likely to differ. As stated by the notifier, this conclusion has been lately challenged by

reanalysis of the microarray data of both studies (Currie *et al* 2014). Lately, two studies provided more evidence for CAR-dependent MoA of propiconazole. First, in their study using CAR-knockout mice Tamura et al (2015) showed CAR to have a crucial role in liver tumor development by three triazoles (cyproconazole, fluconazole, tebuconazole). Although all cyproconazole-induced liver effects were not negated in CAR-knockout mice there were no adenomas. Secondly, a gene expression biomarker signature assessment comparing effects in wild-type and CAR-null mice showed that propiconazole increases liver weights and hepatocyte proliferation in a CAR-dependent manner (Oshida *et al*. 2015). Since the liver responses to propiconazole are very similar to those induced by cyproconazole (CLH-dossier of cyproconazole), these findings on CAR-null mice suggest that CAR-activation has a prime role as a mediator for propiconazole-induced tumourgenesis.

In conclusion, there is clear evidence that CAR receptor activation is involved in tumorigenic action of propiconazole in CD-1 mice. Although it remains equivocal whether this mechanism is the only motivator, recent studies on CAR-null mice suggest that CAR-activation is required for propiconazole-induced hepatocellular tumorgenesis in mice.

4.9.4 Summary and discussion of carcinogenicity

There were no treatment-related neoplasms at any dose level in a two-year combined toxicity and carcinogenicity study in rats (DAR IIA 5.5/02). Although the highest dose (96.46 and 130.63 mg/kg bw/day in males and females, respectively) caused reduced body weight gains and signs of hepatic challenge in both sexes, there was no indication of tumourgenesis. Thus, propiconazole was not considered to be carcinogenic in rats.

Following dietary administration for up to 2 years, high doses of propiconazole resulted in an increased incidence of liver tumours in male CD-1 mice. Incidence of adenomas was statistically significantly increased and slightly above the contemporary historic control range at 107.8 mg/kg/day (DAR IIA 5.5/05) and 344.3 mg/kg/day (DAR IIA 5.5/03,). In male mice at 344.3 mg/kg/day, incidence of liver carcinomas was also significantly increased. In female mice there were no significant differences in liver tumour incidences (DAR IIA 5.5/03).

In addition to fact that significantly increased liver tumour incidences were observed in one species (mice) and in males only, there are other factors decreasing the level of concern for human carcinogenicity. First, increased incidence of carcinomas in male mice was observed only after two years propiconazole treatment, at dose level (344.3 mg/kg/day) clearly exceeding maximum tolerated dose (Table 23). At this dose after one year propiconazole treatment, only a few liver carcinomas (3 vs. 0 in controls, %) were observed (DAR IIA 5.5/03). In the other study (DAR IIA 5.5/05) at the dose considered as MTD (107.8 mg/kg/day), there were no significant differences in the incidences of carcinomas (2%, 6%, 4% and 4%, at 0, 100, 500 and 850 ppm, respectively) after 18 months propiconazole treatment. Thus, increased incidence of malignant tumours was only observed as a consequence of excessive dose at the end of normal lifespan of mice. Secondly, the increased incidence of carcinomas at highest dose was primarily due to an increase in the number of well differentiated hepatocellular carcinomas and there was no significant difference in the morphologic appearance or biological behaviour of the carcinomas observed in the control as compared to the treated groups. Contemporary reference study showed relatively high spontaneous variability in incidences of hepatocellular adenomas (6-18%) and carcinomas (8-16%) in CD mice strain. Thus, propiconazole seems to promote formation of spontaneously occurring liver adenomas, which have low potency to develop malignancy.

Based on histopathological findings and mechanistic studies propiconazole induces liver tumours by a non-genotoxic mechanism which involves CAR activation, induction of mitogenic hepatocyte

proliferation and enlargement, hepatomegaly, and increase of xenobiotic metabolism. The mechanism involves minimal to moderate hepatocellular necrosis. At continuous exposure, preneoplastic lesions and finally hepatocellular tumours are formed. There is clear evidence that CAR receptor activation is involved in tumorigenic action of propiconazole in CD mice. Although it remains equivocal whether this mechanism is the only motivator, recent studies in CAR-null mice suggest that CAR-activation is required for propiconazole-induced tumourgenesis.

4.9.5 Comparison with criteria

According to CLP criteria a substance should be classified in Category 2 (Suspected human carcinogen):

"…when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, ***based on strength of evidence together with additional considerations.*** *Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from* ***limited evidence of carcinogenicity in animal studies****."*

There are no human data for propiconazole. The three available animal studies demonstrate that propiconazole is not carcinogenic in the rat, but at high doses in male mice increases incidences of liver adenomas and carcinomas to levels slightly above contemporary historical control range. However, increased incidence of carcinomas was only observed as a consequence of excessive dose at the end of normal lifespan of mice. Morphology and biological behaviour of these carcinomas did not differ from those of control mice. Thus, propiconazole primary promoted formation of spontaneously occurring liver adenomas, which have low potency to develop malignancy. These factors (possibility of confounding effect of excessive toxicity, reduced tumour latency, spontaneous tumours only at high doses) decrease the level of concern for human carcinogenicity.

In conclusion, propiconazole promoted tumours in single tissue (liver) of single species (mice) in one sex. Treatment-related induction of both benign and malignant tumours in single species and one sex with non-genotoxic mode of action could warrant classification for Category 2 carcinogen. However, the additional considerations above decrease the level of concern for human carcinogenicity. Thus, the data available from the two oncogenic studies in the mouse do not, therefore, support a classification for carcinogenicity for propiconazole.

4.9.6 Conclusions on classification and labelling

CLP: No Classification

RAC evaluation of carcinogenicity
Summary of the Dossier Submitter's proposal One 2-year carcinogenicity study in mice reported incidences slightly above the contemporary historic controls of liver tumours in male CD-1 mice at dose levels of 107.8 and 344.3 mg/kg bw/d. However, the DS observed several factors reducing the relevance of this tumours for humans: i) the effect appeared only in male mice; ii) the effect did not appeared in rats; iii) the tumours appeared only at the end of the two years of exposure and at the maximum

tolerable dose (107.8 mg/kg bw/d) or higher (344.3 mg/kg bw/d); iv) another study showed no significant differences in incidences of liver carcinomas after 18 months of propiconazole exposure; v) a contemporary study showed relatively high spontaneous variability in incidences of hepatocellular adenomas and carcinomas in CD mice; vi) several studies showing a non-genotoxic mechanism involving CAR activation, induction of mitogenic hepatocyte proliferation and enlargement, hepatomegaly, and increase of xenobiotic metabolism, which is of less relevance for humans.

All these considerations reduced the concern of the liver tumours in mice and lead the DS to propose no classification of propiconazole for carcinogenicity.

Comments received during public consultation

Three MSCAs, one manufacturer/company and one industry/trade association supported the 'no classification' proposed by the DS.

One international non-governmental organization (NGO) commented that additional studies, not included in the CLH dossier, related to the potential carcinogenicity of propiconazole were found through a PubMed search and further commented that these publications according to them had far less risk of bias than the toxicity studies performed by Industry. The DS replied that CLP classification is mainly based on intrinsic properties of the substance through studies conducted in accordance with the EU test methods (Regulation 440/2008). The DS also replied that they had reviewed the published scientific literature prior to submission of the CLH proposal and that the classification proposal is based on studies which are considered adequate and reliable for classification purposes.The same NGO also commented that several papers describing a potential mutagenicity of propiconazole had not been taken into account and also questioned the validity of one of the studies where they suspected fraud and non-GLP compliance.

The DS replied to these comments clarifying that the incidences of hepatocellular adenomas in the available studies were 21 versus 40% and 2 versus 20% and that the incidence of hepatocellular adenomas in one study performed *ad hoc* in the same laboratory ranged between 6 and 18%, while this same parameter in another laboratory was essentially identical and ranged between 6 and 18.4%. Thus, according to the DS's opinion, incidences of liver adenomas in propiconazole treated males were statistically significantly increased compared to concurrent controls and slightly above the contemporary historical control range. The DS also clarified that the proposal of no classification for propiconazole is based on several factors that decrease the concern for human carcinogenicity, such as: tumours were found in one species and in males only; malignant tumours (carcinomas) were observed only at a dose level clearly exceeding the maximum tolerated dose at the end of the normal lifespan of mice; and, that propiconazole promoted formation of spontaneously occurring tumours only at high doses.

Regarding the suspicion of GLP fraud the DS consulted the Finnish GLP authorities, EFSA, ECHA and the EU Commission and according to the information from the GLP authority of the country where the testing laboratory is based, the laboratory conducting the 18-month mouse carcinogenicity study was included in their GLP monitoring program in the period of 1997-1999, although this particular mouse study was not audited in the inspections performed in 1996 and 1998.

The DS also reviewed both of the studies conducted on the request of regulatory authorities and studies published in the open scientific literature and found no *in vivo* animal studies showing a tumour profile that would differ from that described in the CLH report.

Finally, the DS reminded the commenter that no evidence of mutagenicity has been observed in the *in vivo* and *in vitro* mutagenicity assays usable for setting classification (those performed according to EU test methods; Regulation 440/2008) and that some of the open scientific literature does not consider propiconazole to act via genotoxic or mutagenic mechanism of action and that the increases in the mutation frequency in the liver is a consequence of oxidative stress.

Assessment and comparison with the classification criteria

2-year chronic toxicity and carcinogenicity study in Sprague Dawley CD rat (DAR IIA 5.5/02)

The main non-neoplastic findings were summarised in the table in the STOT RE section. Mortality rates at the end of the study were as follows: 38, 39, 40 and 31% for males and 53, 45, 45 and 33% in females of dose groups 0, 100, 500 and 2500 ppm, respectively. There was no evidence of treatment-related tumorgenesis.

2-year carcinogenicity study in CD-1 mouse (DAR IIA 5.5/03)

Mortality was high during the study; for males 41, 52, 50 and 64%, and for females 38, 31, 45 and 31%, in the dose groups of 0, 100, 500 and 2500 ppm, respectively. The highest mortality in all groups occurred during the second year of the treatment.

Food consumption of high dose (2500 ppm) males was significantly increased throughout the treatment. Despite this, the body weights and cumulative weight gain of this group remained significantly reduced throughout the study, indicating reduced food utilization. The same effect was apparent, but less marked in high dose females. Absolute body weights of high dose males remained 12-20% lower than controls throughout the study, suggesting that the maximum tolerated dose was exceeded. Cumulative weight gains of intermediate dose (500 ppm) males and females were reduced at the beginning of the study but recovered thereafter.

Histopathological analysis revealed signs of hepatotoxicity in both sexes and increased liver tumour incidence primarily in high dose males. No treatment-related effects were seen in the incidence and distribution of other tumour types, nor in organ weights or tissue histology. To confirm the original diagnosis, the observed tumour response and non-neoplastic changes of the liver were subsequently re-examined (DAR IIA 5.5/04). The morphology and biological behaviour of the liver tumours observed were evaluated according to new (at that time), diagnostic nomenclature and morphologic criteria. All liver specimens from males and females were examined for histopathological changes including neoplastic and non-neoplastic lesions. Proliferative hepatocellular lesions were classified as either foci of cellular alteration, benign (hepatocellular adenoma) or malignant (hepatocellular carcinoma) using the criteria published by the U.S. National Toxicology Program. The hepatocellular carcinomas were classified by degree of differentiation. In addition, all specimens of lung from males were examined for the presence of pulmonary metastasis from malignant hepatocellular neoplasms.

The table below summarises the neoplastic changes found in the carcinogenicity study in mouse. At interim sacrifice (53 weeks), the number of males with hepatocellular adenomas was slightly increased in the 500 ppm group and the number of males with adenomas and/or carcinomas was slightly increased in the high dose group (see table). All carcinomas were well differentiated and there was no evidence of pulmonary metastasis. There was no indication of neoplasia among the females at interim sacrifice.

For the terminal sacrifice (104 weeks) and decedent animals, the incidences of adenomas and/or carcinomas were significantly higher in high dose males than in controls (40% vs. 21% in controls and 80% vs. 51% in controls, respectively). The majority of this response was associated with an increased number of hepatocellular adenomas in high dose males. The slight increase in carcinomas was due to an increase in the number of well differentiated hepatocellular carcinomas relative to the control group. The incidences of moderately well and poorly differentiated carcinomas in treated groups were similar to controls. There was no significant difference in the morphologic appearance or biological behaviour of the carcinomas observed in the control as compared to the treated groups.

The number of adenomas and/or carcinomas was slightly, but not significantly, higher in high dose females compared to controls (9 vs. 6 in controls, number of mice with adenoma and/or carcinoma). No other indication of neoplasia was observed in females.

The total incidences of adenomas, carcinomas and of adenomas and carcinomas in males showed positive linear trend with dose, when all groups were evaluated (method by Peto *et al.*, 1980). When the high dose group was excluded from the analysis, there was no evidence of a linear trend.

No treatment-related effect was seen in the incidence and distribution of other tumour types.

Table: Neoplastic changes in the carcinogenicity study in mouse. Incidences are based on re-examination of liver micropathology (DAR IIA 5.5/04).

	Dietary concentration of propiconazole (ppm)							
	Males				**Females**			
	0	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
Interim sacrifice								
No. animals examined	11	11	11	9	12	11	11	12
Adenoma [a]	1/1	0/0	4/4	4/3	0/0	0/0	0/0	0/0
Carcinoma well differentiated [a]	0/0	0/0	0/0	3/3	0/0	0/0	0/0	0/0
No. of mice with only adenoma	1	0	4	1	0	0	0	0
No. mice with at least one carcinoma	0	0	0	3	0	0	0	0
Adenoma + carcinoma	1	0	4	4	0	0	0	0
Terminal sacrifice and decedents								
No. examined	53	53	51	55	52	53	53	52
Adenoma [a]	25/18	18/11	20/15	68/35	6/5	0/0	2/2	13/8
Carcinoma well differentiated [a]	10/10	6/5	5/5	23/17	1/1	0/0	0/0	2/2
moderately well differentiated [a]	4/4	4/3	9/7	7/6	0/0	1/1	0/0	1/1

poorly differentiated [a]	3/3	2/2	3/3	3/2	0/0	0/0	0/0	0/0
No. mice with only adenoma	11 (21%)	7	9	**22* (40%)**	5	0	2	6
No. mice with at least one carcinoma	16	9	13	22	1	1	0	3
Adenoma + carcinoma	27 (51%)	16	22	**44** (80%)**	6	1	2	9
Total								
No. examined	64	64	62	64	64	64	64	64
No. mice with only adenoma	12 (19%)	7	13	**23* (36%)**	5	0	2	6
No. mice with at least one carcinoma	16 (25%)	9	13	25 (39%)	1	1	0	3
Adenoma + carcinoma	28 (44%)	16	26	**48*** (75%)**	6	1	2	9

[a] Total count of tumours/no. mice with tumours
* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 (pairwise Fisher' Exact Test)

In conclusion, in males the highest dose (2500 ppm) was above the maximum tolerated dose, based on increased mortality (64% compared to 41% in controls), decreases in absolute body weight (11-16% lower than controls), body weight gains (20-38% lower than controls), and evidence of hepatotoxicity. At this dose an increased incidence of liver tumours was observed (adenoma and/or carcinoma 80% vs. 51% in controls) at the end of the 104 weeks. The majority of this response was associated with an increased number of hepatocellular adenomas and the slight increase in carcinomas was primarily due to an increase in the number of well differentiated hepatocellular carcinomas. Signs of hepatotoxicity were observed also in high dose females, but there were no significant increases in liver tumour incidences.

18-months carcinogenicity study in CD-1 mouse (DAR IIA 5.5/05)

This study was conducted for additional mouse oncogenicity study since the high dose (2500 ppm) in the first study was considered to be excessively toxic. No significant clinical signs, behavioural changes or effects on survival were observed, although mortality was high, more than 60%, in all groups during the study.

The table below shows the incidences of neoplastic lesions found in liver (no other organ or tissues were microscopically assessed). A significant increase in adenomas and combined adenoma and carcinoma was found at the highest dose.

Table: Neoplastic findings in liver in 18-month study in mice.

	Dietary concentrations of propiconazole (ppm)			
	0	**100**	**500**	**850**
Hepatocellular adenoma	1 (2%)	0	3	**10** (20%)**
Hepatocellular carcinoma	1	3	2	2
Total hepatocellular tumours	2 (4%)	3	5	**12** (24%)**

Spontaneous liver tumours in CD-1 mice

A contemporary study was conducted in the same laboratory to collect reference control data of in-life parameters and post-mortem findings, including the incidence of liver tumours from CD-1 mice over a period of 18 months under standard laboratory conditions. Five groups per sex with 80 mice per group were used. Incidences of hepatocellular adenoma, adenocarcinoma and combined adenoma + carcinoma ranged between 6 and 18%, between 8 and 16% and between 14 and 30%, respectively (table below).

Table: Incidences of liver tumours in 18-month reference study in CD-1 mouse.

	Group 1	Group 2	Group 3	Group 4	Group 5
Number examined	50	50	50	50	50
Adenoma	9 (18%)	7(14%)	3 (6%)	4 (8%)	7 (14%)
Adenocarcinoma	6 (12%)	8 (16%)	5 (10%)	4 (8%)	7 (14%)
Combined: adenoma + adenocarcinoma	15 (30%)	12 (24%)	7 (14%)	7 (14%)	13 (26%)

The notifier also reported historical control data on CD-1 males from Charles River Laboratory showing a historical control range of 6.0-18.4% for hepatocellular adenomas and 0-12% for hepatocellular carcinomas in four studies with 199 control animals and a mean adenoma incidence of 10.8% with 12 studies with a total of 770 controls.

In conclusion, the incidences of adenomas (36% in the 2-year study at exposure level higher than the maximum tolerable dose and 20% in the 18-month study at exposure level below the maximum tolerable dose) were slightly above the contemporary historical control incidence range (6-18%) from the same laboratory, whereas the combined incidence of adenomas and carcinomas in the 18-month study (24%) was within the contemporary historical control range (14-30%).

Mechanism of action based on constitutive androsterone receptor (CAR) activation

Additional studies to investigate the MoA for the propiconazole-induced liver tumours in mice have been conducted. These studies were conducted to determine if propiconazole exert its liver carcinogenicity via the activation of the constitutive androsterone receptor (CAR), i.e. the phenobarbital mode of action (MoA). The conclusions from the studies are briefly summarised below. For detailed study descriptions, see Background document.

According to Elcombe *et al*. (2014), the CAR-mediated pathway includes the following key and associated events (figure below). Activation of CAR receptor (key event 1) and altered expression of CAR-responsive genes (key event 2),followed by liver enzyme induction (CYP), increased liver weight, hepatocyte hypertrophy (associated events) and increased cell proliferation (key event 3), which leads to clonal expansion leading to altered foci (key event 4) and formation of adenomas and carcinomas (key event 5).

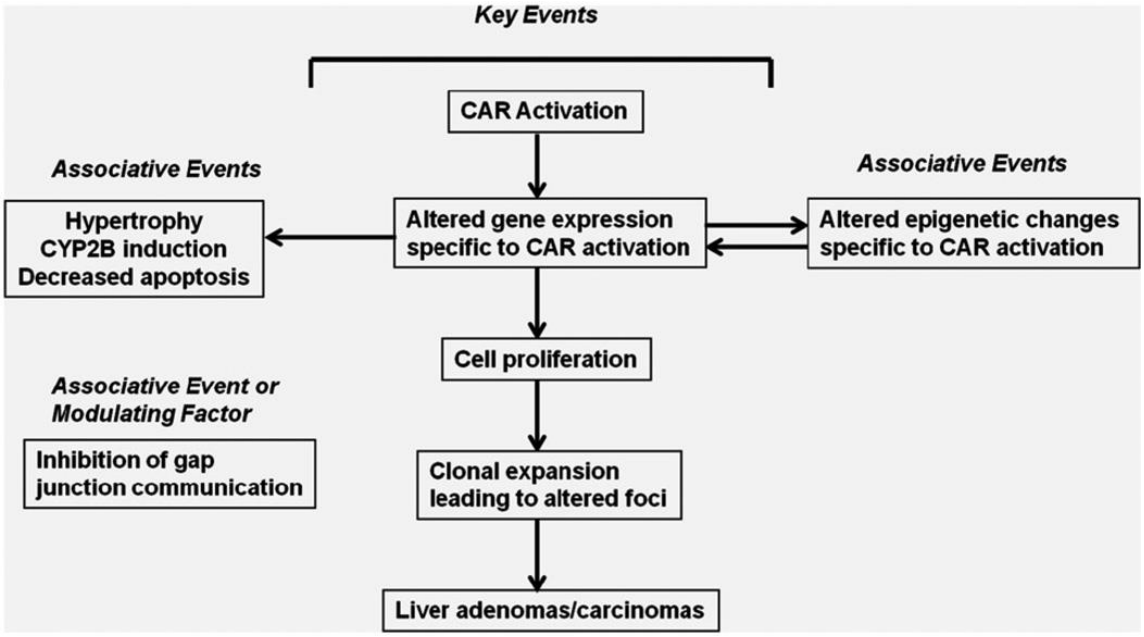


Figure: Mechanism of action rodent liver tumour formation exerted through CAR activation. Taken from Elcombe *et al.*, (2014).

Study 1: The effect of propiconazole on drug metabolizing enzymes in the livers of Tif:RAIf rats and Tif:MAGf mice (DAR II 5.8.6/02)

Male Tif:RAIf rats and Tif:MAGf mice were treated with 0, 20, 80, 160 or 320 mg/kg bw/d by gastric intubation during 14 consecutive days. The animals were sacrificed after the termination of the treatment period and concentrations of the following xenobiotic enzymes were determined in microsomal fractions: Cytochrome P-450, activity of ethoxycoumarin O-deethylase in the presence or in the absence of monooxygenase inhibitors, epoxide hydrolase and UDP-glucuronyltransferase. Glutathione S-transferase with 1-chloro-2,4-dinitrobenzene as substrate was determined in cytosolic fractions, and the activity of glutamyltranspeptidase in rat liver supernatants.

Electron microscopy of histological slides revealed marked proliferation of the smooth endoplasmic reticulum membranes of hepatocytes in both species at the daily dose of 320 mg/kg bw. Statistically significant increases in practically all xenobiotic enzymes were noted in both species, usually at 80 mg/kg bw/d and higher concentrations, with a somewhat more pronounced effect in rat. Especially the enzymes known to be structurally associated with the endoplasmic reticulum were increased by propiconazole treatment. The activity of microsomal epoxide hydrolase was clearly lower in mice than in rats, whereas activities of UDP-glucuronosyltransferase and glutathione S-transferase were significantly higher.

Propiconazole caused clearly discernible changes in the ultrastructural organisation of hepatocytes and was an efficient inducer of xenobiotic metabolism in both rat and mouse. The profiles of liver enzyme induction in rat and mouse by propiconazole were found to be different in some respects, especially concerning activities of cytochrome P-450 enzymes, epoxide hydrolase, and glutathione S-transferase. The possible explanation to the observed

differences in hepatic tumour formation between the two species and the sex differences noted in mice could be based on the observed differences in xenobiotic metabolism.

Study 2: Tumour promotion study with propiconazole in Tif:RAIf rat (DAR II 5.8.6/01).

Male and female Tif:RAIf rats pre-treated with 15 mg/kg N-nitrodiethylamine (i.p.) or vehicle were used for testing the tumour promoting activity of propiconazole administered in feed. The vehicle control and N-nitrosodiethylamine (DENA) treatment groups were further divided into three dietary treatment groups receiving: 1) control diet, 2) 500 ppm phenobarbital (positive control), or 3) 2000 ppm propiconazole. The duration of the study was 56 days. Sections of liver were examined for histopathological changes by staining with haematoxyline and eosine (HE) and periodic acid Schiff (PAS). Hepatocyte structure in histopathological sections was studied by detecting cellular ɣ-glutamyltransferase (ɣ-GT) activity. The ɣ-GT stained sections were used to determine the presence and histological nature of focal and diffuse ɣ-GT-positive changes.

Propiconazole or phenobarbital alone enhanced the formation of ɣ-GT positive foci smaller than 0.01 mm^2 by increasing their number but not their size. When propiconazole or phenobarbital were given after pre-treatment with DENA, more and larger foci were observed. More foci smaller than 0.01 mm^2 were found after 56 days of treatment with 2000 ppm propiconazole than with 500 ppm phenobarbital in DENA pre-treated rats. Similar numbers of foci larger than 0.01 mm^2 were found after treatment with propiconazole and phenobarbital.

The conclusion from this study was that propiconazole acts as a promoter of proliferative changes in rat liver at dietary concentrations of 2000 ppm.

Study 3: Assessment of hepatic cell proliferation in male CD-1 mice (DAR IIA/5.8)

The study was designed to characterize the extent and time dependence of hepatocyte proliferation at tumorigenic doses of propiconazole. Eight groups of five young adult male CD-1 mice were fed diet containing 0, 850 or 2500 ppm propiconazole (equivalent to 0, 127, 353 or 139 mg/kg bw/d) or 850 ppm phenobarbital (139 mg/kg bw). Interim sacrifices were performed at 1, 2, 3, 4, 7, 14, 28 and 60 days. To examine hepatocyte cell proliferation, each animal received a single i.p. injection of BUdR (100 mg/kg bw) 2 h before sacrifice. Cell proliferation was assessed by BUdR-immunohistochemistry/image analysis.

Rapid time- and dose-related increases in absolute and relative liver weights were observed in propiconazole treated animals. The maximum increase in absolute liver weight was 147% in the low dose propiconazole group and 241% in the high dose group, compared to controls. The duration of the weight gain period was 3 days for the low dose and 14 days for the high dose. Treatment with phenobarbital resulted in a liver weight maximum of 175%, compared to the control, and the weight gain reached a plateau within 4 days.

Hepatocellular hypertrophy was found in all low and high propiconazole dose animals immediately after treatment. At 850 ppm the mean severity, increasing with time, ranged from minimal (1-day treatment) to moderate/marked (28- and 60-day treatment). At 2500 ppm the mean severity of hypertrophy ranged from minimal (day 1) to marked (days 28 and 60). Treatment with 850 ppm phenobarbital led to a progression of hepatocellular hypertrophy similar to the one observed in the 2500 ppm propiconazole group.

Minimal hepatocellular necrosis was observed in several animals of the 850 ppm propiconazole group, especially after prolonged treatment. Minimal/moderate necrosis of the liver was found in almost all 2500 ppm propiconazole and 850 ppm phenobarbital treated animals.

Mitotic activity of hepatocytes was increased in most treated animals during days 2-4 of exposure. The maximum incidence and severity was observed on day 2 for both propiconazole dose levels (moderate/marked; all animals) and for phenobarbital (marked; all animals).

Cytoplasmic vacuolation was divided into panlobular cytoplasmic vacuolation and centrilobular cytoplasmic vacuolation. Panlobular vacuolation (minimal) was observed in 1–3 animals per group during days 3–7 in the propiconazole groups, and in 2 animals of the day 3 phenobarbital group. Centrilobular vacuolation was observed towards the end of the treatment, and with a much higher incidence and severity (practically all animals from day 7 to 60; minimal to marked) in 2500 ppm propiconazole treated animals than in 850 ppm propiconazole (days 4 [1 mice] and 60 [3 mice]; minimal) or phenobarbital (day 28 [1 mice]; minimal) treated animals.

The 850 ppm propiconazole group exhibited significantly increased mean BUdR labelling indices on days 1-4. Peak values of relative mean labelling indices were found on days 1 and 2; the values returned to control level on day 7. The 2500 ppm propiconazole group showed significantly increased mean labelling indices on days 1–7. The peak relative mean labelling index value was noted on day 2. From day 14 on, the values dropped to values slightly above control levels. Treatment with 850 ppm phenobarbital resulted in statistically increased mean labelling indices on days 1-7. A peak relative mean labelling index value was found on day 2. From day 14 on the values dropped to values slightly above control levels.

Treatment with propiconazole at 850 and 2500 ppm for up to 60 days caused a prominent, time- and dose-related hepatomegaly. The liver enlargement was caused by a sharp and transient induction of hepatocellular proliferation and to a time- and dose-related increase in the severity of hepatocellular hypertrophy. In general, the temporal pattern of propiconazole induced hepatocyte proliferation was the same as for phenobarbital, suggesting that propiconazole is a phenobarbital-like mitogen in the male mouse liver.

<u>Study 4: Effects on biochemical parameters in the liver following administration to male CD-1 mice (DAR IIA/5.8)</u>

Liver enzyme induction was investigated in male CD-1 mice after administration of tumorigenic doses of propiconazole. Groups of 6 young adult male CD-1 mice were treated for 14 days with propiconazole at dietary doses of 0, 850 and 2500 ppm, and with the reference compound phenobarbital at 850 ppm, corresponding to mean daily doses of 0, 149, 578 and 145 mg/kg bw/d, respectively.

The major treatment-related biochemical alterations in the liver at treatment with propiconazole at 2500 ppm comprised of increases in total microsomal cytochrome P450 content, cytochrome P450 isoenzyme Cyp2b-10-dependent O-dependentylation of 7-pentoxyresorufin, Cyp2a-dependent coumarin 7-hydroxylase, testosterone oxidation at several positions, content of barbiturate- and steroid-inducible microsomal cytochrome P450

isoenzymes of subfamilies Cyp2b and Cyp3a (table below). These findings indicate a phenobarbital type of induction of xenobiotic metabolising enzymes of the liver.

Table: Biochemical parameters in the liver of CD-1 male mice.

	Relative amount of activity (% of control)		
	Phenobarbital	**Propiconazole**	
	850 ppm	**850 ppm**	**2500 ppm**
Strong to moderate effects with propiconazole			
Microsomal cytochrome P450 content	239	300	389
Microsomal pentoxyresorufin O-depentylase (PROD)	3534	3024	5524
Microsomal coumarin 7-hydroxylase	480	534	2384
Microsomal testosterone 2β-hydroxylation	466	298	531
Microsomal testosterone 6α-hydroxylation	779	502	715
Microsomal testosterone 6β-hydroxylation	500	366	524
Microsomal testosterone 15β-hydroxylation	526	316	977
Microsomal testosterone 16β-hydroxylation	5.7[1]	5.2[1]	6.4[1]
Microsomal testosterone oxidation to androstenedione	179	589	652
Total microsomal testosterone oxidation	356	440	555
Microsomal epoxide hydrolase	179	172	321
Immunoblot Goat anti Rat CYP2B1 (Cyp2b) Band 1	2743	2608	3049
Immunoblot Goat anti Rat CYP2B1 (Cyp2b) Band 2	581	810	579
Immunoblot Goat anti Rat CYP2B1 (Cyp2b) Band 3	351	439	617
Immunoblot MAb p6 (Cyp3a)	577	658	1068
Slight effects with propiconazole			
Microsomal ethoxyresorufin O-deethylase (EROD)	232	219	388
Microsomal testosterone 2α-hydroxylation	0[1]	2.7[1]	2.7[1]
Microsomal testosterone 16α-hydroxylation	194	214	262
Microsomal lauric acid 11-hydroxylase	271	267	305
Microsomal lauric acid 12-hydroxylase	163	153	161
Microsomal UDP-glucuronosyltransferase (UDPGT)	156	156	139
Cytosolic glutathione S-transferase (GST)	187	158	184
No effects with propiconazole			
Microsomal testosterone 7α-hydroxylation	229	175	125
Microsomal protein content	97	109	113
Cytosolic protein content	92	97	94

Immunoblot MAb d15 (Cyp1a)	89	124	89
Immunoblot MAb clo4 (Cyp4a)	129	140	157

[1] Absolute values, control value below limit of detection

Treatment with propiconazole at 850 ppm generally resulted in a lower but still clear induction for all biochemical parameters, which demonstrates a dose-dependency for the liver enzymatic induction (table above).

The lack of an effect of propiconazole on the content of microsomal cytochrome P450 isoenzymes of subfamilies Cyp1a and Cyp4a as well as only minor effects on microsomal cytochrome P450 isoenzyme Cyp1a-1-dependent O-deethylation of 7-ethoxyresorufin and Cyp4a-dependent 12-hydroxylase of lauric acid exclude the mode of action as a 3-methylcholanthrene- or peroxisome proliferator-type inducer.

Treatment with the reference compound phenobarbital at a dose of 850 ppm resulted in a similar induction profile as with propiconazole. The induction by phenobarbital was quantitatively similar to that seen with propiconazole at 850 ppm. Differences to propiconazole were a lower extent of induction of androstenedione formation from testosterone and the lack of induction of testosterone 2α-hydroxylation.

In this study, sub-chronic treatment of male mice with 850 and 2500 ppm propiconazole or 850 ppm phenobarbital caused strong and qualitatively similar induction effects on liver weight and biochemical liver parameters. Thus propiconazole was found to be a strong phenobarbital-type inducer of xenobiotic metabolising enzymes in the mouse liver.

Study 5: Cytochrome P450 2b, 3a and DNA-synthesis induction in cultured male CD-1 mouse hepatocytes (dRAR B6.8.2.2)

This study investigated the ability of propiconazole to induce Cyp2b10 and Cyp3a11 expression and changes in cell proliferation (measured as the change in replicative DNA synthesis) in primary male CD-1 mouse hepatocyte cultures. Phenobarbital sodium salt and epidermal growth factor were included as positive controls for induction of Cyp isoforms and cell proliferation, respectively. Hepatocytes were exposed to propiconazole at 6 concentrations (0.2, 1, 5, 25, 125 and 500 µM), to phenobarbital at 3 concentrations (10, 100 and 1000 µM) or vehicle (0.5% v/v dimethyl sulfoxide) alone for 96 hours.

Treatment with 125 and 500 µM propiconazole resulted in significant cytotoxicity with reductions of intracellular ATP levels to 37.3% and 0.8% of control, respectively (figure below). Thus, replicative DNA synthesis as well as Cyp2b10 and Cyp3a11 mRNA data could either not be measured at these concentrations or were consequently excluded from data analysis. Treatment with 0.2 µM and 1 µM propiconazole resulted significant (90% and 92.2% of control, respectively), but less pronounced reductions in intracellular ATP. The tested phenobarbital concentrations (up to 1000 µM) did not induce cytotoxicity (figure below).

Treatment with 5 µM propiconazole resulted significant increase in expression of Cyp2b10 mRNA (see figure), but not protein, and slight increase in Cyp3a11 expression (see figures). Treatment with 25 µM propiconazole resulted in statistically significant increases in replicative DNA synthesis and expression of Cyp3a11 mRNA and protein. A slight decrease in Cyp2b10 expression was observed at this concentration of propiconazole. Treatment with 100 µM

phenobarbital resulted significant increase in replicative DNA synthesis and 1000 µM phenobarbital resulted increased expression of Cyp2b10 and Cyp3a11.

In this study it was concluded that 5 µM propiconazole resulted in the induction of Cyp2b10 mRNA levels, while Cyp3a11 mRNA was increased at 25 µM and both concentrations (5 and 25 µM) induced cell proliferation in mouse hepatocytes consistent with activation of CAR.

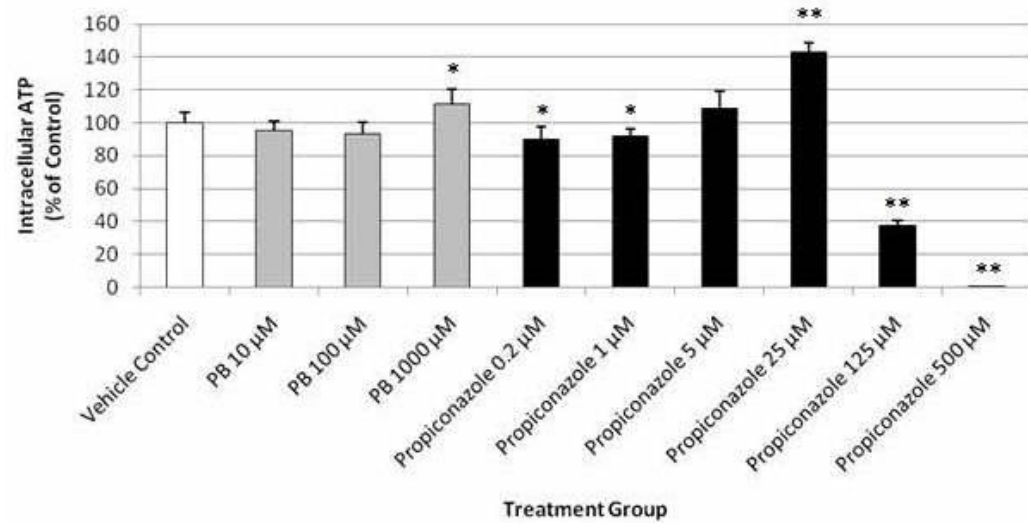


Figure: Intracellular ATP in mouse hepatocytes. Values are Mean ± SD (n = 6). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$; **$p<0.01$. PB = Phenobarbital.

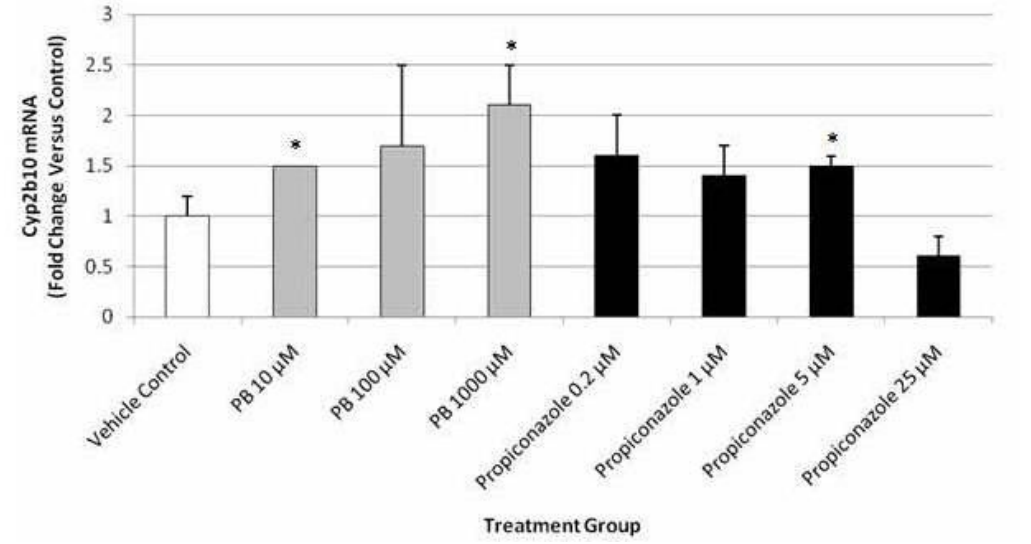


Figure: Cyp2b10 mRNA expression. Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$. PB = Phenobarbital.

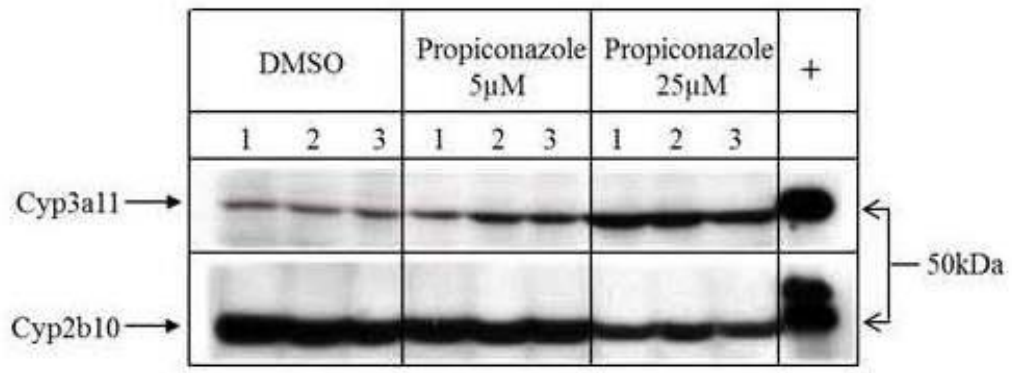


Figure: Cyp3a11 and Cyp2b10 protein expression. Mouse hepatocyte proteins (40 µg) were resolved on 7.5% polyacrylamide gels and transferred to PVDF membranes using standard methods. Membranes were probed with either rabbit anti-CYP3A (CH32, diluted 1/4000) or rabbit anti-CYP2B (CH4, diluted 1/4000); the secondary antibody was HRP-linked donkey anti-rabbit IgG (GE Healthcare, diluted 1/4000). Recombinant mouse Cyp3a11 and Cyp2b10 were used as positive controls for Cyp3a11 and Cyp2b10, respectively.

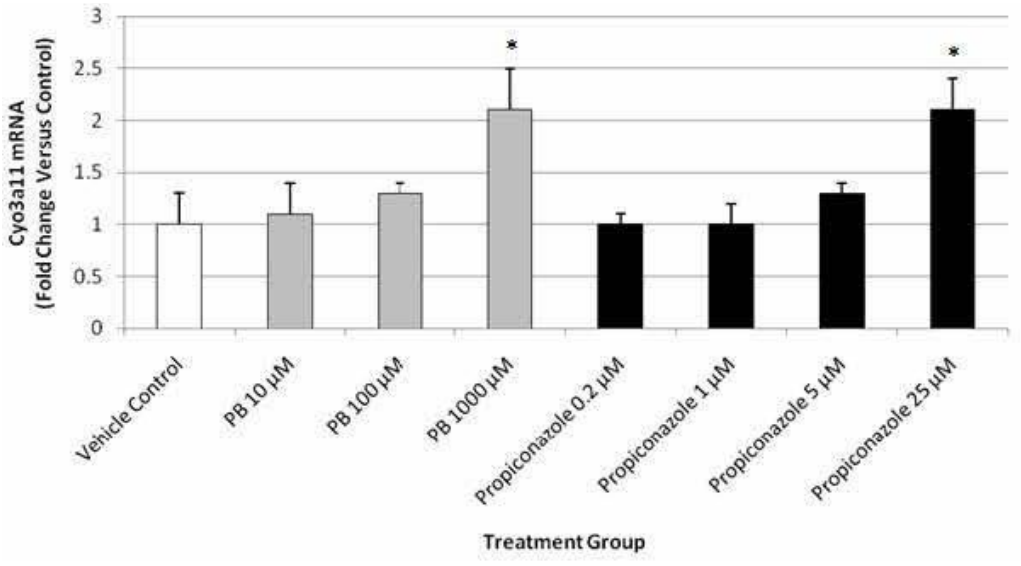


Figure: Cyp3a11 mRNA expression. Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05. PB = Phenobarbital.

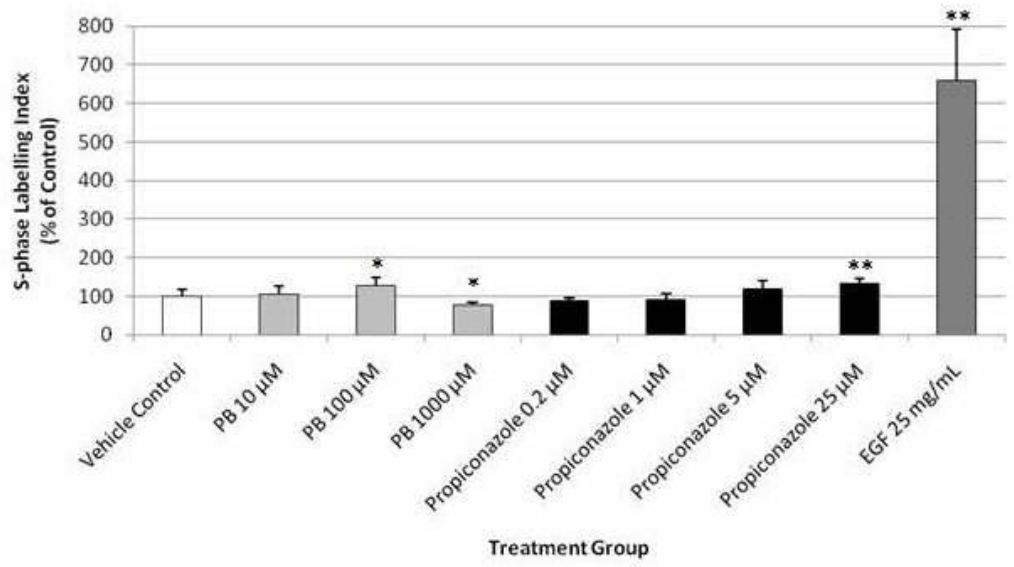


Figure: Replicative DNA synthesis (S-phase). Values are Mean ± SD (n = 5). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05; **p<0.01. PB = Phenobarbital. EGF = epidermal growth factor (positive control).

Study 6: Cytochrome P450 2B, 3A and DNA- synthesis induction in cultured male human hepatocytes (dRAR, B.6.8.2.3)

This study investigated the ability of propiconazole to induce CYP2B6, CYP3A4 and cell proliferation (change in replicative DNA synthesis) in isolated male human hepatocyte cultures. Phenobarbital sodium salt and epidermal growth factor were included as positive controls for induction of CYP isoforms and cell proliferation, respectively. Hepatocytes were exposed to propiconazole at 6 concentrations (0.2, 1, 5, 25, 125 and 500 µM), to phenobarbital at 3 concentrations (10, 100 and 1000 µM) or to vehicle (0.5% v/v dimethyl sulfoxide [DMSO]) alone for 96 hours.

Treatment with 5, 25, 125 and 500 µM propiconazole resulted in statistically significant lower intracellular ATP, with levels being reduced to 90, 55, 4 and 0.4% of control, respectively (see figure). The cytotoxicity observed following treatment with 125 and 500 µM was considered excessive, and therefore replicative DNA synthesis, CYP2B6 and CYP3A4 mRNA data could either not be measured at these concentrations or were excluded from data analysis. Treatment with 1000 µM phenobarbital reduced intracellular ATP levels to 89% of control values (see figure).

Treatment with EGF resulted in significant increase in replicative DNA synthesis to 14.6-fold control, indicating that the hepatocytes could proliferate following exposure to proliferative stimuli (see figure). Neither propiconazole nor phenobarbital induced increases in replicative DNA synthesis (S-phase of the cell cycle), indicating that neither compound induced cell proliferation.

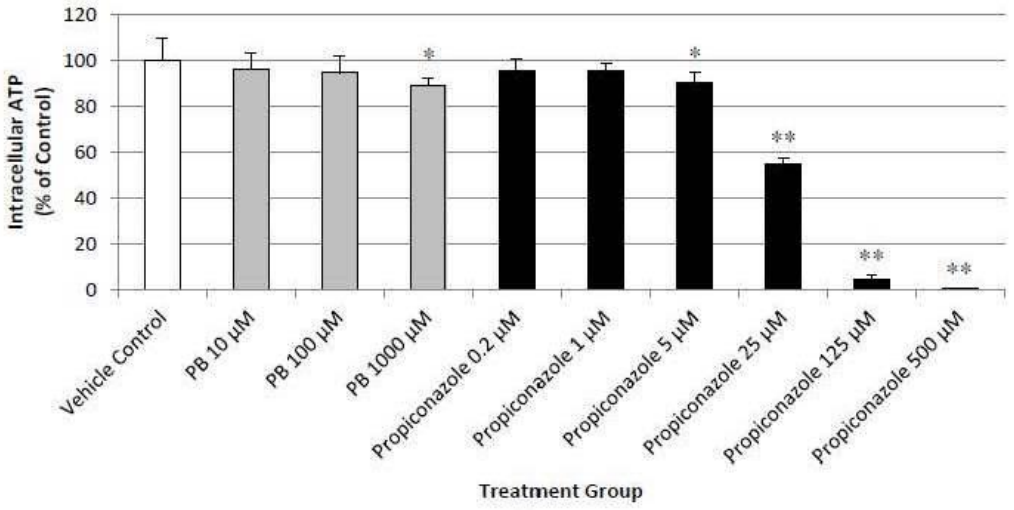


Figure: Intracellular ATP in human hepatocytes. Values are Mean ± SD (n = 6). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$;**$p<0.01$. PB = Phenobarbital.

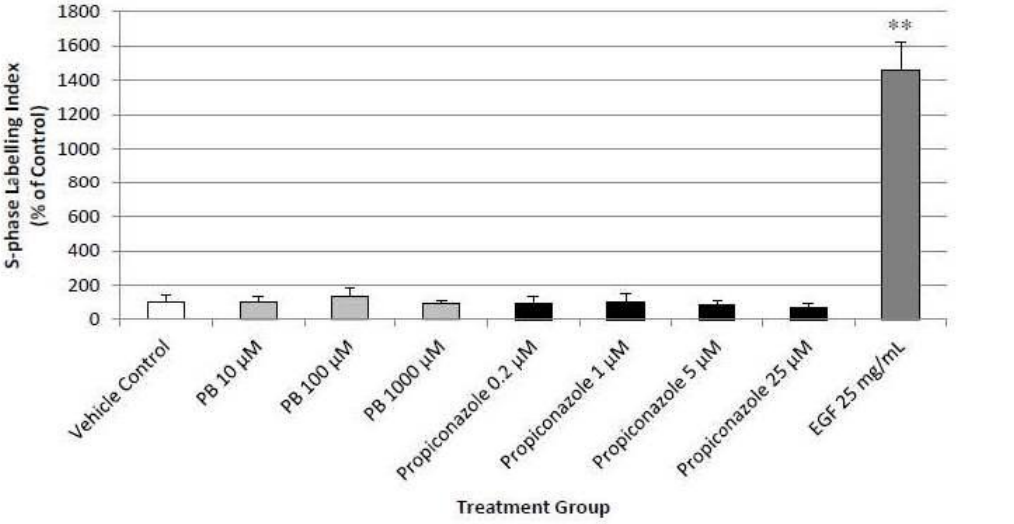


Figure: Replicative DNA synthesis (S-phase). Values are Mean ± SD (n = 5). A Student's t-test (2-sided) was performed on the results; *statistically different from control $p<0.05$;**$p<0.01$. PB = Phenobarbital. EGF = epidermal growth factor (positive control).

Treatments with 100 µM and 1000 µM phenobarbital and 5 µM and 25 µM propiconazole resulted in statistically significant increases in both CYP2B6 and CYP3A4 mRNA (figures below). These increases indicate increased expression of the cyp2b6 and cyp3a4 genes. Hence, CYP3A4 mRNA induction was clearly lower at 25 µM compared to 5 µM propiconazole.

This possibly reflects increasing cytotoxicity. CYP3A4 mRNA levels were significantly increased also after treatment with 10 µM phenobarbital and 1 µM propiconazole. Treatment with 5 and 25 µM propiconazole resulted in increased CYP3A4 protein amount compared to the control group.

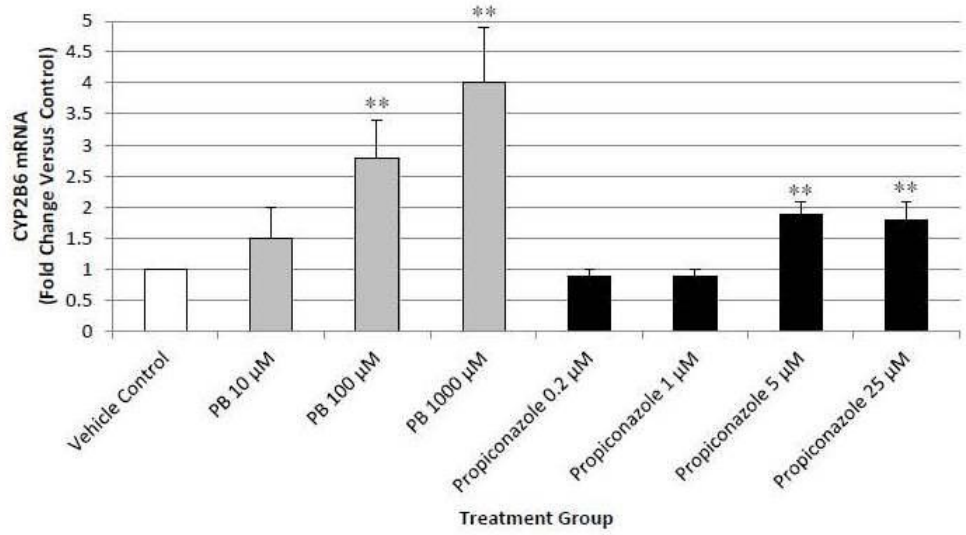


Figure: CYP2B6 mRNA expression. Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05;**p<0.01. PB = Phenobarbital.

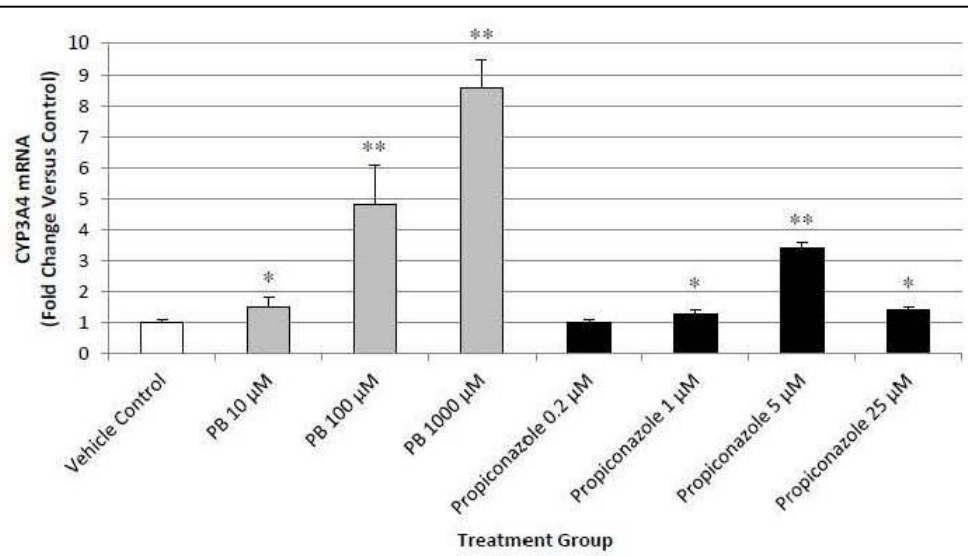


Figure: CYP3A4 mRNA expression. Values are Mean ± SD (n = 3). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05;**p<0.01. PB = Phenobarbital.

Phenobarbital and propiconazole induced CYP2B6 and CYP3A4 transcripts without affecting cell proliferation in human hepatocytes. This is consistent with species differences in CAR and PXR receptors between humans and rodents.

Study 7: CAR3 direct activation assay with mouse, rat and human CAR (dRAR, B.6.8.2.4)

Propiconazole was tested for its ability to directly activate the constitutive androstane receptor (CAR, NR1I3) in a reporter assay. After a suitable expression time, the cells were incubated with propiconazole at concentrations of 1, 3, 10, and 30 µM. The direct CAR activator meclizine was also incubated at these same concentrations, and model direct-acting substrates for mouse, rat or human CAR were each incubated at a single concentration.

A strong concentration-dependent activation of rodent CAR3 by propiconazole was observed, with up to 40-fold activation of mouse CAR3 and up to 60-fold activation of rat CAR3. In contrast, the human CAR3 response was only statistically significant at 30 µM, the highest dose tested, and this response only represented a 3-fold activation above solvent control (see figure below).

The model activators CITCO (prototype human CAR ligand), TCPOBOP (prototype mouse CAR ligand) and clotrimazole (CLOT) produced robust responses in human, mouse and rat CAR3 constructs, respectively. Meclizine was also tested and produced a concentration-dependent response that was much more marked with mouse CAR3 than with human or rat CAR3.

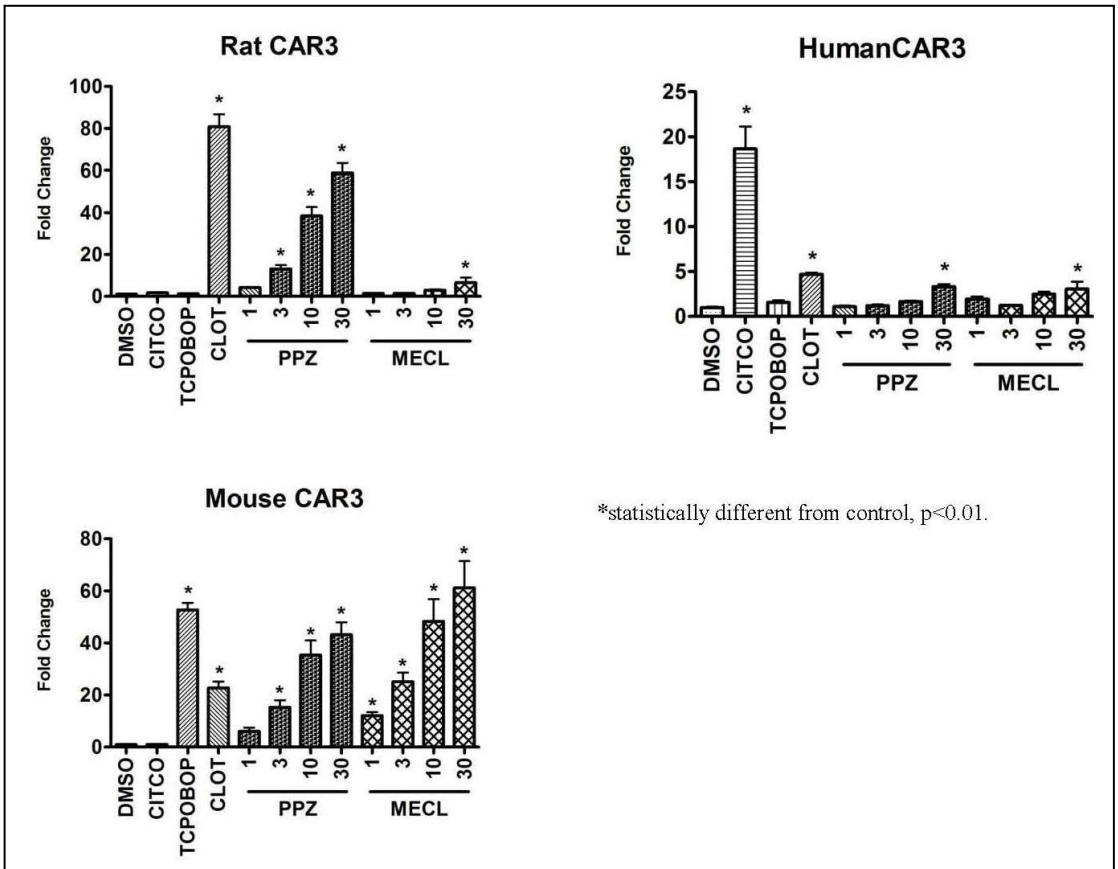


Figure: A comparison of CAR activation by propiconazole and positive control compounds in rat, mouse and human CAR3 (fold change relative to DMSO control). CITCO = human CAR ligand. TCPOBOP = mouse CAR ligand. CLOT = rat CAR ligand. MECL = Meclizine (direct CAR activator).

This study showed that propiconazole is a direct CAR activator in mouse, rat and human and under the conditions of this assay the activation of rat CAR with propiconazole was strongest,

whereas responsiveness of human CAR to propiconazole was much weaker than mouse and rat CAR. This suggests a quantitative difference between rodent CAR and human CAR with respect to their direct activation by propiconazole.

Overall discussion of mechanistic studies based on constitutive androsterone receptor (CAR) activation

According to Elcombe *et al*. (2014) the CAR-mediated pathway for induction of liver tumours consists in seven key events (depicted in a figure above). The table below overalls the experimental evidences for supporting such key events available for three differences species.

Table: Summary of evidences for the different key and associative events of a CAR-mediated induction of liver tumours in mice, rats and humans.

Key event	Mice	Rat	Humans	Study
CAR activation	YES	YES	YES	dRAR, B.6.8.2.4
Altered gene expression	Cyp 2b10 Cyp 3a11	Not determined	CYP 2B6 CYP 3A4	dRAR B6.8.2.2, dRAR, B.6.8.2.3
CYP induction	YES	YES	Not determined	DAR II 5.8.6/02, DAR IIA/5.8, dRAR B6.8.2.2
Increase liver weight	YES	YES	Not determined	DAR II A 5.3.1/01, DAR IIA 5.5/02, DAR II A 5.3.2/03, DAR IIA 5.3.2/04, DAR IIA 5.5/03, DAR IIA 5.5/05, dRAR B.6.3.3.1.2, DAR II 5.8.6/02, DAR II 5.8.6/01, DAR IIA/5.8, DAR IIA/5.8
Liver hypertrophy	YES	Not determined	Not determined	DAR IIA/5.8
Cell proliferation	YES	Not determined	**NO**	DAR IIA/5.8, dRAR B6.8.2.2, dRAR, B.6.8.2.3
Hepatic foci alteration	YES	YES	Not determined	DAR II 5.8.6/01, DAR IIA 5.5/02, DAR IIA 5.5/03
Tumour formation	YES	NO	Not determined	DAR IIA 5.5/02, DAR IIA 5.5/03, DAR IIA 5.5/05

The table above shows that all the key events in the development of liver tumours through CAR activation has been experimentally supported in mice and most of them in rat. RAC notes that one potential gap in the mechanistic information was the decrease of apoptosis as consequence of alterations in gene expression. The CLH dossier contains an Annex with a document submitted by the notifier with an assessment of the MoA for liver tumours induced by propiconazole using the framework developed by IPCS and ILSI/HESI. In this document it is stated that *"increased expression of pro-proliferative and anti-apoptotic gene Gadd45β"* was detected after exposures to 850 ppm propiconazole. However, there is no reference to support this information and the details were not contained in the CLH-report and consequently this information could not be assessed by RAC. Nevertheless, RAC considers that the overall picture of the available experimental information makes plausible that the

mechanism of liver carcinogenesis induced by propiconazole in mouse was based on CAR-activation.

There were severe interspecies quantitative differences in the activation of CAR by propiconazole, specifically, 30 µM propiconazole is able to activate mouse, rat and human CAR by 60, 40 and 3-fold of solvent controls, respectively. These differences might be responsible of the fact that propiconazole failed to induce liver tumours in rat, while did it in mouse.

RAC notes two critical differences between mouse and humans. These differences were: i) the activation of human CAR is around 20 times lower than the activation of mouse CAR; and, ii) cell proliferation could not be detected through replicative DNA synthesis in human hepatocytes, while did in mouse hepatocytes. These two differences play in favour of lack of relevance of this CAR activation mechanism for humans.

Potential alternative mode of action for liver carcinogenesis induced by propiconazole

DNA reactivity and mutagenicity

Propiconazole was negative in wide array of *in vitro* and *in vivo* genotoxicity assays.

Peroxisome proliferation

Propiconazole produced little or no increase in lauric acid 12-hydroxylase activity and Cyp4a levels of protein in liver fractions of treated mice. Both of these markers are greatly increased by peroxisome proliferators, which suggest that propiconazole is not a peroxisome proliferator.

Aromatic Hydrocarbon Receptor P450 induction

Propiconazole did not produce a large increase in EROD activity nor an increase in Cyp1a protein levels in liver microsomes of treated mice. Both of these markers are greatly increased by aromatic hydrocarbon receptor activators, which suggest that propiconazole is not an aromatic hydrocarbon receptor activators.

Estrogenic stimulation

Propiconazole did not bind to the oestrogen receptors at most concentrations tested, and appeared to severely disrupt the assay at very high concentrations (10^{-3} M). Propiconazole was negative for estrogenic effects in an uterotrophic *in vivo* assay in the ovariectomized rat. In combination with the lack of effects on oestrogen-sensitive tissues in the wider toxicology database, the weight of evidence indicates that propiconazole does not show estrogenic potential. RAC notes that all this information was cited in the CLH report as described in several references but that were no accessible to RAC.

Cytotoxicity and regenerative hyperplasia

The evidence does not support a finding of regenerative hyperplasia, which is the causal key event required for carcinogenesis to be produced as a secondary consequence of hepatotoxicity.

Cell proliferation caused by propiconazole was transient and can be contrasted with the sustained regenerative cell proliferation and development of long-term fibrosis seen with classical hepatotoxic agents that induce regenerative hyperplasia such as chloroform and carbon tetrachloride. As an example, an increase in liver cell proliferation was observed for up to 159 days of treatment in a study with chloroform in B6C3F1 mice, but no cell proliferation was observed beyond 7 days in the current studies with propiconazole.

In the *in vivo* mouse studies, a limited amount of hepatic necrosis (single cell or focal/multi-focal) plus chronic inflammatory cell infiltration were observed, which is in contrast with the pattern of effects seen with classic cytotoxic carcinogens that cause a diffuse necrosis in the liver that progressed to regenerative hyperplasia, as is the case of chloroform.

In conclusion, the weight of evidence shows that a MoA involving cytotoxicity and a subsequent sustained regenerative cell proliferation is not operative with propiconazole. RAC notes that the information cited in the CLH report regarding chloroform were no accessible to RAC.

Statins/altered cholesterol biosynthesis

Propiconazole was not designed to inhibit HMG-CoA reductase so this MoA is unlikely to be operating. Nevertheless, plasma cholesterol levels were decreased in mice by propiconazole treatment. The sites of action in the cholesterol synthesis and metabolism pathway that are theorized to cause this effect are thought to be different from the statins. Experiments with another triazole fungicide, cyproconazole, have shown that the effect of lower plasma cholesterol at a tumorigenic dose of 200 ppm was completely blocked in mice lacking the CAR receptor. CAR receptor activation has been shown to play a role in regulation of lipogenesis, β-oxidation of fatty acids, gluconeogenesis and cholesterol/bile acid metabolism. Therefore it is likely that an alteration in cholesterol metabolism is also a consequence of CAR activation by propiconazole.

Additional considerations for classification

The Guidance on the Application of the CLP Criteria establishes certain important factors which may be taken into consideration when assessing the overall level of concern. These factors are displayed and discussed in the table below.

Table: Some important factors which may be taken into consideration when assessing the overall level of concern of the propiconazole-induced tumours.

Tumour type:	Liver tumours
Multi-site responses:	No (only liver)
Progression of lesions to malignancy:	Malignancy appeared only above the maximum tolerable dose
Reduced tumour latency:	No (the malignant tumours occurred at a later stage of the study)
Whether responses are in single or both sexes:	Single sex (males)
Whether responses are in a single species or several species:	Single species (mice)
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:	Not noted
Routes of exposure:	Oral (relevant for human)
Comparison of absorption, distribution, metabolism and excretion between test animals and humans:	Not known

The possibility of a confounding effect of excessive toxicity at test doses:	Carcinomas appeared in concurrence with liver toxicity, only adenomas were seen below the maximum tolerable dose.
Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity:	Potentials modes of action have been discussed above, but it is plausible that the MoA was through CAR activation, which is of low relevance for humans.

Comparison with the criteria

A substance can be classified as carcinogenic Category 1A when it is known to have carcinogenic potential for humans on the basis of human evidence. There is no information about the potential carcinogenicity of propiconazole for humans and therefore Category 1A is not supported.

A substance can be classified as carcinogenic Category 1B when it is presumed to have carcinogenic potential for humans on the basis of animal evidences, while Category 2 is reserved for substances suspicious to be carcinogenic on the basis of evidences not sufficiently convincing to classify as Category 1.

RAC notes that there are two different studies in mouse demonstrating that propiconazole is able to induce hepatocarcinogenicity, which in principle is enough to propose classification in Category 1B. However, RAC notes other factors that considerably reduce the level of concern regarding the propiconazole carcinogenicity for humans. These factors are:

- The tumours appeared only in one species (mice), tissue (liver) and sex (males);
- The incidence of hepatocellular carcinomas exceeded those of the controls only at doses clearly exceeding maximum tolerable doses in the 2-year carcinogenicity study causing 40% reduction body weight gain, while at the maximum tolerable dose in the 18-month carcinogenicity study the incidence of carcinomas were similar to that reported for control;
- There was no significant difference in the morphological appearance or biological behaviour of the carcinomas observed in the control when compared to the treated groups;
- No significant incidence of carcinomas was reported after 53 weeks of exposure at doses above the maximum tolerable dose, which suggest a long time of latency;
- The incidences adenomas (20%) at maximum tolerable dose in the 18-month carcinogenicity study was only slightly above the incidence of spontaneous adenomas (6-18%) reported in the same laboratory for a contemporary study;
- The incidences of carcinomas (4%) and combined adenomas plus carcinomas (24%) at maximum tolerable dose (in the 18-month carcinogenicity study) were within the incidences of spontaneous carcinomas (8-16%) and spontaneous adenomas plus carcinomas (14-30%) reported in the same laboratory for a contemporary study;
- Experimental evidence supporting a MoA for the induction of liver tumours in male mice attributable to CAR activation, with quantitative interspecies differences in response to CAR activation between mice and humans depicted in a figure above
- Low plausibility for other potential alternative MoA for liver carcinogenesis induced by propiconazole.

RAC also notes uncertainties in the available database, as the lack of information about how many independent hepatocyte cultures were used in the dRAR B6.8.2.2 (mouse) and specially in dRAR B6.8.2.2 (human) or the absence of data with CAR-knock-out mouse.

Nevertheless, the overall available information suggests that the liver tumours found in mice after exposure to propiconazole are not of concern for humans and RAC agrees with the DS that **no classification for carcinogenicity of propiconazole is warranted.**

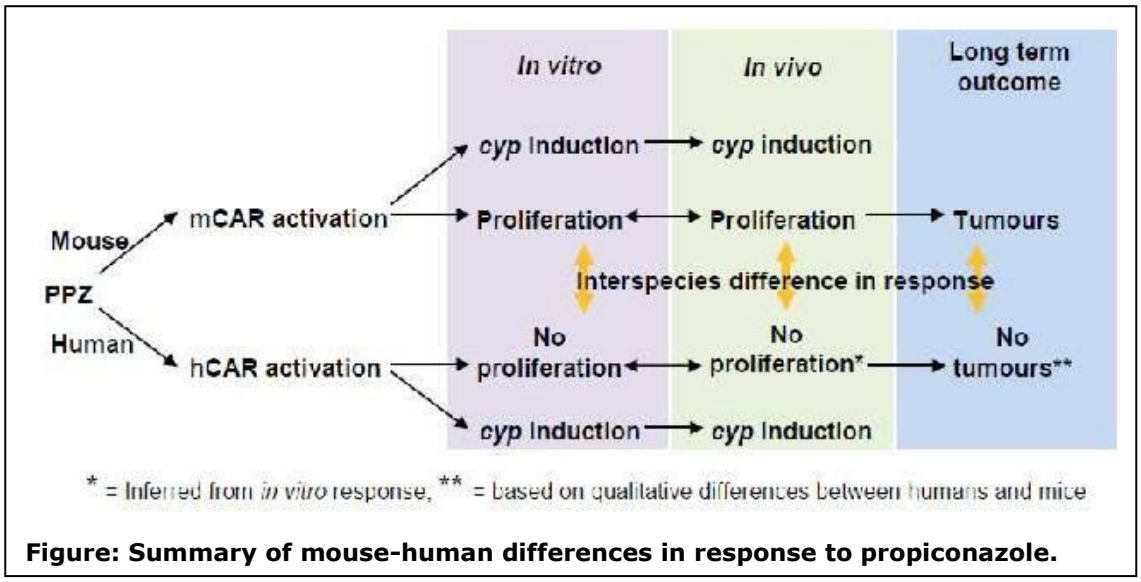


Figure: Summary of mouse-human differences in response to propiconazole.

4.10 Toxicity for reproduction

Table 34: Summary table of relevant reproductive toxicity studies

Method **Guideline** **GLP**	**Species** **Strain** **Sex** **no/group**	**Doses** **Route** **Exposure period**	**Results/ Remarks**	**Reference**
Fertility Two generation reproduction study FIFRA Guidelines draft 1982 Draft OECD 418, 1981 Equivalent or similar to OECD Guideline 416 GLP Acceptable	Rat, Charles River CD strain 15 males and 30 females	Purity: 89.7% 0, 100, 500 and 2500 ppm Oral, diet Pre-mating and through mating, gestation and lactation of two litters per generation (approx. 30 weeks for each parental generation, continuous)	NOAEL parental toxicity: 100 ppm (avg intake 8.4 mg/kg bw/day males and 9.7 mg/kg bw/day females). Decreased body weight and food consumption, and liver histopathological changes at 500 and 2500 ppm. NOAEL reproduction and offspring toxicity: 500 ppm (avg. intake 48.8 mg/kg/bw/day males and 43.7 mg/kg bw/day females). Reduced litter size, number of pups delivered viable and increased number of runt pups at 2500 ppm (avg. intake 214.9 mg/kg/bw/day in males and 242.9 mg/kg/bw/day in females). Reduced testes plus epididymides weights of male offspring at 2500 ppm.	DAR IIA 5.6.1/01 Key Study
Developmental toxicity Teratology study OECD Guideline 414, 1981 (Prenatal Developmental Toxicity Study) GLP Acceptable	Rat Crl:COBS CD (SD) BR VAF/PLUS) 30 females	Purity: 88 %, technical grade 0, 30, 90, 360/300 mg/kg/day (nominal conc.) The high dose was reduced to 300 mg/kg/day due to severe signs of maternal toxicity Oral, gavage Vehicle: 3% corn starch containing 0.5% Tween 80 Daily treatment on days 6-15 of gestation	NOAEL maternal toxicity: 30 mg/kg bw/day. Decreased body weight gain and food consumption at higher doses. Marked maternal toxicity was observed at 360/300 mg/kg/day. NOAEL developmental toxicity: 30 mg/kg bw/day. Increased incidence of cleft palate at 300 (2/285 fetuses) and 90 mg/kg/day (1/302 fetuses). Increased incidences of visceral and skeletal variations at 90 and 360/300 mg/kg/day.	DAR IIA 5.6.2/01 Key Study

Method **Guideline** **GLP**	**Species** **Strain** **Sex** **no/group**	**Doses** **Route** **Exposure period**	**Results/ Remarks**	**Reference**
Teratology study Modified OECD Guideline 414, 1981 (Prenatal Developmental Toxicity Study) GLP Acceptable	Rat Crl:COBS CD (SD) BR VAF/PLUS 200 females	Purity: 88% Oral, gavage 0, 300 mg/kg/day (nominal conc.) Vehicle: 3% corn starch containing 0.5% Tween 80 Daily treatment on days 6-15 of gestation	The study was designed to clarify equivocal cause of cleft palates, not to establish NOAEL. At the tested dose (300 mg/kg bw/day): Severe maternal toxicity (reduced absolute body weights, weight gain and food consumption; severe clinical signs of toxicity and low incidence of mortality). Reduced fetal weight and viability. Increased incidence of cleft palate 2/2064 (0.097%) pups in 2/158 litters.	DAR IIA 5.6.2/02
Teratology study OECD Guideline 414, 1981 (Prenatal Developmental Toxicity Study) EPA OPP 83-3 GLP Acceptable	Rabbit New Zealand White 19 females	Purity: 88% Oral, gavage 0, 100, 250, 400 mg/kg/day (nominal conc.) Vehicle: 3% corn starch containing 0.5% Tween 80 Daily treatment on days 7-19 of gestation	NOAEL maternal toxicity: 100 mg/kg bw/day. Reduced food consumption and body weight gain at 250 and 400 mg/kg bw/day. NOAEL reproduction and developmental toxicity: 250 mg/kg bw/day. Increased incidence of resorptions, abortions and early deliveries and increased incidence of fully formed 13th rib at 400 mg/kg/day.	DAR IIA 5.6.2/03 Key Study

4.10.1 Effects on fertility

4.10.1.1 Non-human information

In **a two-generation reproduction study** propiconazole was administered in the diet (*ad libitum*) at concentrations 0, 100, 500 and 2500 ppm to groups of 15 male and 30 female Charles River CD rats over two generations (DAR IIA 5.6.1/01). The main deviations to current OECD 416 were: oestrus cycle and sperm parameters were not determined, developmental landmarks of the offspring including parameters of sexual maturation were not evaluated, food consumption was only determined during pre-mating period, only brain, ovary and testes weights were determined.

The study was started when F0 parental animals were 5 weeks old. F0 animals were treated continuously with propiconazole over 12 weeks pre-mating period, and thereafter over two generations. In matings (20-days period) one male was cohabited with two females. Females were examined daily to detect breeding (copulatory plug or sperm-positive vaginal smears). When breeding was conformed females were transferred to individual cages. Males were rotated among the females

at 10-day intervals. Each female was paired with a maximum of 2 different males. Conception was confirmed by the observation of a vascular membrane in the vagina and/or the detection of progeny by palpation. Females produced two litters in both generations. Matings for second litters (b) were initiated approximately two weeks after weaning of first litters (a) and same procedures were followed. F0 and F1 parental animals were weighed weekly during 12 weeks pre-mating period, and parental females thereafter monthly. Females which failed to conceive, deliver viable progeny, or retain progeny throughout the lactation period were weighed monthly among with males until their termination. Food consumption was determined weekly for F0 and F1 parental animals during the pre-mating period. Animals were observed at least twice per day for mortality, morbidity, and overt signs of toxicity.

The sexes and numbers of pups delivered viable, stillborns, and found partially cannibalized were recorded. Each pup was examined for developmental anomalies at birth and again at weaning. The numbers of pups surviving to lactation days 4, 7, 14, and 21 (weaning) were recorded. Pup weights were determined on lactation days 0, 4, 7, and 21 for all surviving progeny, but pups were not identified within the litter with unique identification number prior to weaning. Pups were examined twice per day for mortalities. Pup number in each litter was adjusted to 8 (optimally 4 females and 4 males).

Gross necropsy and histopathological examinations were performed to all parental animals. From the offspring, 10 males and 10 females were randomly selected from each dose group for gross pathological and histopathological examination. In addition, progeny found dead during the lactation period, and progeny with apparent developmental anomalies were examined. Weights of brain, ovaries and testes (with epididymides) were determined. Histopathology was limited to liver, ovaries, prostate, seminal vesicles, testes, uterus and vagina. In addition, tissues and organs appearing abnormal we examined. F0 parental animals were sacrificed approximately at the age of 37 weeks (after 32 weeks treatment), F1 generation parental males approximately at the age of 33-35 weeks and F1 generation parental females at the age of 35-39 weeks.

Results

No consistent treatment-related ante mortem observations were noted. Three F0 parental females died prior to final sacrifice. A 100 ppm female was found dead during the premating period, a 500 ppm female was found dead during the rest period between the F_{1a} and F_{1b} litters, and a 2500 ppm female was found dead after parturition of her F_{1b} litter. Two F1 2500 ppm females died during parturition of their F_{2a} litters. One male control animal was sacrificed in a moribund condition during the F_{1b} matings and one 500 ppm male was sacrificed in a moribund condition during the F_{2b} matings (a cage injury).

Food consumptions of F0 and F1 parental females from 2500 ppm groups were significantly reduced compared to controls throughout the premating period. Food consumptions of F1 females with diets containing 500 ppm or 100 ppm propiconazole were also reduced (500 ppm group significantly reduced compared to controls at week 4, and 100 ppm group at weeks 4 and 8). F0 and F1 generation males from 2500 ppm groups also exhibited reduced food intake (significant reductions at weeks 1 and 7, and 2, 6, 10; respectively). Body weights of F0 and F1 parental females receiving 2500 ppm propiconazole were significantly lower compared to controls throughout the treatment. The pre-mating weight gain and the total weight gain of F0 and F1 females were also significantly reduced (Table 35). F1 parental females in the 500 ppm group had significantly lower body weights than controls at weeks 10 and 11, and significantly reduced pre-mating weight gain was also noted (Table 35). Body weights of F0 generation males receiving 500 and 2500 ppm of propiconazole were

slightly reduced compared to controls throughout the treatment (statistically significant difference at week 2 for 2500 ppm males). Body weights of F1 generation males receiving 2500 ppm of propiconazole were reduced compared to controls throughout the treatment (statistically significant differences at weeks 9-11).

Table 35: Body weight gains (g) of parental animals

	Dietary concentration of propiconazole							
	Males				**Females**			
	0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
Pre-maiting weight gain (weeks 1-12)								
F0	369.0	350.7	339.9	339.7	172.7	173.1	168.4	137.0**
F1	434.3	440.9	439.7	396.1	225.8	220.6	206.2**	191.6**
Total weight gain								
F0	509.5	500.9	479.8	481.2	252.0	240.9	254.4	193.4**
F1	593.0	615.5	612.3	550.2	313.4	306.8	293.5	254.3**

** statistically significant difference P<0.01

Table 36: Body weights (g) of dams over gestation and lactation periods (week 13 forward)

Week (approximate)	**Dietary concentration of propiconazole**							
	F0				**F1**			
	0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
16	296.0	305.4	318.4	269.6	284.3	285.3	286.3	249.6
20	311.9	308.0	312.5	271.3**	317.7	304.9	301.7	262.3
24	351.8	354.9	365.7	286.3	306.3	329.1	323.3	275.5
28	361.0	371.4	374.1	305.0	327.3	343.3	337.1	280.1*
32	-	-	-	-	336.7	337.7	322.2	283.7**
Final body weight	360.5	350.1	363.7	301.7**	353.1	350.1	336.6	287.9**

* statistically significant difference P<0.05, ** statistically significant difference P<0.01

Gross pathological examination of the parental animals from F0 and F1 generations revealed no untoward treatment related findings. Treatment-related changes were found in the liver of both F0 and F1 generation females and males. Swelling of hepatocytes in both periportal and centrilobular areas of the liver, was observed in the livers of male and female F0 and F1 rats. The swelling involved a mild increase in the size of hepatocytes with an apparent increase in cytoplasmic volume and compression of adjacent hepatic sinusoids. The incidence and severity of cellular swelling increased with dose. The incidence of this lesion was statistically significant for the 500 ppm F_0 and F_1 males and for the 500 ppm F1 females, and also for the 2500 ppm F0 and F1 parents (both male and female). The incidence of clear-cell change in the liver also increased at 2500 ppm in the F0 males and in the F1 males and females and at 500 ppm in the F1 males. The clear-cell change consisted of both individual and small clusters of hepatocytes with marked cytoplasmic vacuolation scattered throughout the liver sections.

Table 37: Histopathological changes in the liver of parental animals and progeny

Generation	Dietary concentration of propiconazole							
	Males				Females			
	0 ppm	100 ppm	500 ppm	2500 ppm	0 ppm	100 ppm	500 ppm	2500 ppm
Hypertrophy								
F0	7/15	3/15	**13/15***	**14/15***	4/30	3/29	6/30	**29/30***
F1	0/15	1/15	**5/15***	**15/15***	0/30	2/30	**15/30***	**29/30***
F1b	2/10	1/10	2/10	**10/10***	1/10	1/10	2/10	**8/10***
F2b	0/10	0/10	2/10	**10/10***	0/10	0/10	1/10	**9/10***
Vacuolation								
F0	0/15	2/15	3/15	**14/15***	1/30	1/29	1/30	1/30
F1	2/15	5/15	**8/15***	**11/15***	2/30	4/30	7/30	**10/30***
F1b	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
F2b	0/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10

* statistically significant difference P<0.05

Statistical analysis of reproductive data (mating, fertility, gestation, female fertility and male fertility index, average gestation length) revealed no significant reductions in these parameters. Delivery and population data (the mean numbers of pups delivered, delivered viable, stillborn and partially cannibalized at birth, the numbers of survived pups during the lactation period) obtained for the groups of dams exposed to propiconazole were comparable to the control dams during both the F_{1a} and F_{1b} litters. During the F_{2a} litter, the numbers of pups delivered, delivered viable and surviving to lactation day 4, were significantly reduced for the 2500 ppm dams (Table 38). During the F_{2b} litter the pup survival indices at lactation days 7, 14, and 21 were significantly reduced for the 2500 ppm group dams (Table 38).

In F_{1a} and F_{2a} litters, progeny of dams exposed to 2500 ppm had significantly lower body weights than controls on lactation days from 4, 7, 14 and 21 (Table 38). Body weights of 2500 ppm F_{1b} progeny were significantly lower than those of the control progeny on lactation days 14 and 21. Body weights of 2500 ppm F_{2b} progeny were significantly lower than the control progeny on lactation days from 0, 4, 7 and 21. The body weight data obtained for the 100 and 500 ppm progeny revealed inconsistent statistically significant reductions and increases in comparison to controls with equivocal biological significance (Table 38). The birth weights for the 100 ppm F_{2b} progeny were reduced, but there was an increase in the mean numbers of pups per litter delivered by 100 ppm dams (Table 38).

Table 38: Selected litter data

Litter	Day[a]	Dietary concentration of propiconazole (ppm)							
		Mean number of live pups				Mean pup body weight (g)			
		0	**100**	**500**	**2500**	**0**	**100**	**500**	**2500**
F1a	0	12.2	11.7	11.2	11.8	6.1	6.0	6.1	6.2
	4	11.0	11.4	11.0	11.4	8.9	8.8	9.2	**8.1****
	7	6.8	7.4	7.5	7.7	14.6	**13.0****	15.2	**12.3****
	14	6.8	7.2	7.5	7.4	28.2	**25.4****	**26.9***	**21.9****
	21	6.8	7.2	7.4	7.4	48.0/46.1	**44.1/42.5****	47.7/44.9	**35.8/34.7****
F1b	0	11.4	12.7	13.2	12.4	6.1	6.0	**6.4****	6.1
	4	10.3	12.3	12.9	12.1	8.8	9.2	**9.5****	8.6
	7	6.4	7.7	7.8	7.4	13.4	14.5	**14.5***	13.3
	14	6.3	7.7	7.7	7.2	25.7	**27.7***	**28.0****	**22.9****
	21	6.3	7.7	7.7	7.2	44.3/40.1	46.1/43.5	47.7/43.5	**35.6/32.5****
F2a	0	12.5	13.0	10.8	**9.2****	5.4	5.3	**5.7***	5.4
	4	12.0	11.6	10.4	**8.1****	8.5	**7.9****	8.3	**7.5****
	7	7.8	7.2	7.2	6.5	13.7	13.3	13.0	**10.8****
	14	7.8	7.2	7.0	6.2	25.6	25.4	25.2	**20.0****
	21	7.8	7.1	7.0	6.2	43.8/41.1	42.5/40.4	42.5/39.8	**31.5/30.2****
F2b	0	12.8	13.4	12.7	11.3	5.8	**5.4****	**5.5****	**5.4****
	4	12.5	13.2	12.5	10.6	8.8	8.6	8.5	**7.3****
	7	7.7	8.0	7.8	**6.2***	14.6	14.7	13.8	**10.9****
	14	7.7	8.0	7.8	**5.7****	29.4	28.7	**26.8****	**21.9****
	21	7.7	8.0	7.8	**5.7****	48.8/46.2	48.6/46.6	**45.1/42.3****	**36.7/33.3****

Day 21 pup body weights are males/females ** statistically significant difference P<0.01, * P<0.05

Absolute brain and testes (including epididymides) weights of the 2500 ppm group of F_{1a} male progeny were significantly reduced in comparison to controls. Absolute testes weights and testes to brain weight ratios of 2500 ppm F_{2a} males were also significantly reduced compared to controls. The testes findings were not confirmed by microscopy, since histopathological examinations were only performed on F_{1b} and F_{2b} litters. A significant increase in the brain to body weight ratio was noted in the 2500 ppm F_{2b} males and a significant reduction in the brain weight was noted in the 2500 ppm F_{2b} females.

Examination of the external structural development of the progeny did not reveal any statistically significant differences. Two 100 ppm F_{1a} anomalous pups (anurous and club limbs; cleft lip), one 2500 ppm F_{1a} anomalous pup (a partially opened left eyelid and a left eye which was smaller than normal), two 2500 ppm F_{1b} anomalous pups (a domed forehead [the brain was dilated] and eyes which appeared smaller than normal; a left eye which was enlarged with an opacity) and one 2500 ppm F_{1b} pup with an unopened eyelid (the eye was however not missing, and apparently of normal size) were obtained from dams treated with propiconazole. One stillborn 500 ppm F_{2a} pup exhibited agnathia and possible exencephaly (dam cannibalized top of head). One 100 ppm F_{2b} pup displayed clubbed limbs, shortened torso and a shortened tail. One 500 ppm F_{2b} pup kept its left eyelid closed (the left eye was shrunken in size). Number of runt pups was significantly increased in the 2500 ppm F_{2b}

progeny. Swelling of hepatocytes was found in the liver of the F_{1b} and F_{2b} weanlings (histopathological examination was not performed on F_{1a} and F_{2a} litters). The incidence of this lesion was statistically significant at 2500 ppm (Table 37) .

Conclusions

The NOAEL in this two generation reproduction study in rats using dietary administration of propiconazole was 100 ppm (app. 8.4 mg/kg bw/day males and 9.7 mg/kg bw/day females), based on histopatological changes in the liver of parents at 500 and 2500 ppm. Reproductive effects, consisting of increased number of runt pups, reductions in pup weights, in numbers of delivered pups, pups delivered viable, and in pup survival were observed at 2500 ppm (app. 214.9 mg/kg/bw/day males and 242.9 mg/kg/bw/day females). In addition, testes plus epididymides weights were reduced in F_{1a} and of F_{2a} pups exposed to 2500 ppm. Histopathological changes of the liver were observed also in the offspring exposed to 2500 ppm propiconazole. There were no significant effects on reproductive indexes (mating, fertility, gestation) in this study. No effects on reproduction and postnatal development were observed at a dietary concentration of 500 ppm (app. 48.8 mg/kg/bw/day males and 43.7 mg/kg bw/day females).

4.10.1.2 Human information

No information available.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

The potential of propiconazole to induce prenatal developmental toxicity was investigated in two rat studies and one rabbit study**. In a rat developmental toxicity study (DAR IIA 5.6.2/01)** propiconazole (technical grade, purity 88%) was administered to three groups of female rats at dose levels of 30, 90 or 360 mg/kg bw/day (the high dose was reduced to 300 mg/kg bw/day due to severe signs of maternal toxicity). The doses were not corrected to correspond to the pure compound. A total of 120 sexually mature virgin female Crl:COBS CD (SD)BR VAF/PLUS rats were mated with 60 sexually mature males of the same strain. The day mating was confirmed was designated as day 0 of gestation. The test article was administered once daily by gastric intubation as a suspension in 3% cornstarch containing 0.5% Tween 80 from day 6 through day 15 of gestation at a volume of 10.0 ml/kg/day. Control animals received an equivalent volume of vehicle. All suspensions were found to be within ± 22% of the nominal concentration. The highest dose was reduced to 300 mg/kg bw/day on the sixth day of dosing due to severe maternal toxicity. The dams were observed daily for changes in appearance and behavior. The body weights were recorded on gestational days 0, 6, 8, 12, 16 and 20. Food consumptions were recorded for a weekly period from day 0 to day 6, and daily thereafter throughout the gestation. The dams were necropsied on day 20 of presumed gestation. The uteri were weighed and corpora lutea, live fetuses, dead fetuses and intrauterine resorption sites were counted. The fetuses were sexed and numbered in order of their positions in the uterus. Viable fetuses were weighed and fetuses were examined for gross abnormalities. Approximately one half of the fetuses from each litter were examined for visceral and another half for skeletal abnormalities. For dams histopathological examination was carried out to selected tissues. This study pre-dates the current (2001) version of the OECD 414 guideline which requires a longer treatment period (from implantation to one day prior to the day of scheduled kill).

Results

Signs of severe compound-related toxicity were observed in 360 mg/kg bw/day dams during the first week of treatment. The toxic signs included significant increase in lethargy, ataxia and salivation, and signs of rales, prostration, hypothermia and bradypnea. The toxic signs decreased immediately following the lowering of the dose level to 300 mg/kg bw/day on the sixth day of dosing. Due to the length of the mating period and the random assignment of pregnant animals to the dose groups, the exact dosing regimen for each animal cannot be confirmed from the report. However, four animals received 5 doses of 360 mg/kg bw/day, nine animals 4 doses of 360 mg/kg bw/day, nine animals 3 doses of 360 mg/kg bw/day and two animals two doses of 360 mg/kg bw/day before the dose was decreased to 300 mg/kg bw/day. This decrease was accomplished by reducing the administered volume of the intubation in this dose group from 10.0 ml/kg/day to 8.3 ml/kg/day. The scheme is summarised in the following table to illustrate the timing of the change of dose level.

Table 39: Summary of the dosing regimen for high dose animals

No. animals	Dose level of propiconazole	
	360 mg/kg bw/day	300 mg/kg bw/day
4/24	5 doses (days 6-10)	5 doses (days 11-15)
9/24	4 doses (days 6-9)	6 doses (days 10-15)
9/24	3 doses (days 6-8)	7 doses (days 9-15)
2/24	2 doses (days 6-7)	8 doses (days 8-15)

There were no treatment-related clinical observations in dams of the 30 mg/kg bw/day or 90 mg/kg bw/day group. Body weight gain and food consumption were significantly reduced in the intermediate and high dose dams following the onset of dosing but both parameters returned to control levels around gestation day 11 (Table 40 and Table 41). For the overall study period (days 0-20), body weight gain was comparable to controls in all treatment groups. There were no significant differences in absolute body weights between the control and any of the treatment groups during the study. Necropsy or histopathological examination revealed no treatment related lesions in dams.

Table 40: Maternal body weight gain (g)

Day of gestation	Dose level of propiconazole (mg/kg bw/day)			
	0	30	90	360/300
0-6	29.05	32.00	32.41	28.09
6-8	7.14	3.76 (47%↓)	3.14* (56%↓)	2.68* (62.5%↓)
8-12	16.59	18.43	16.71	14.23
12-16	23.09	22.19	23.91	23.27
16-20	49.27	51.33	59.68	59.91
0-20	125.14	127.71	133.05	128.18
0-20#	57.23	52.24	56.11	50.95

*Statistically significant difference to control at $P < 0.05$, Days 0-20# = Body weight gain excluding uterus, placentas and fetuses weight

Table 41: Maternal food consumption (g) - selected

Days of gestation	Dose level of propiconazole (mg/kg bw/day)			
	0	30	90	360/300
0-6	19.45	20.57	20.75	19.96
6-7	21.00	21.90	18.83	18.82
7-8	21.00	21.43	20.32	16.82* (20%↓)
8-9	22.18	22.24	19.68* (16%↓)	18.58* (16%↓)
9-10	21.77	23.19	20.82	16.68* (23%↓)
10-11	22.77	22.38	19.82* (13%↓)	20.36
11-12	22.36	23.71	22.95	21.82
19-20	25.00	25.62	26.45	26.27

* Statistically significant difference to control at $p < 0.05$

Despite maternal toxicity, all reproduction parameters remained similar in all groups (Table 42). There were no significant differences in fetal weights between treated groups and controls. External and visceral examinations revealed one fetus in intermediate group to have cleft lip and cleft palate, micromelia and a club foot, and other fetus from different litter in the intermediate group to have cleft lip. In the high dose group one fetus had cleft palate, and another fetus from different litter had anasarca, cleft palate, hydromelia and protruding tongue. Significant increases in the incidences of short and absent renal papilla(e) and dilated ureters were detected in fetuses of the high dose group. Significant increases in the incidences of rudimentary ribs and non-ossified sternebrae were observed in fetuses of the high and intermediate dose groups (Table 42).

Table 42: Summary of reproductive parameters and foetal findings

	propiconazole (mg/kg bw/day)			
	0	**30**	**90**	**360/300**
Number of pregnant females (% of mated)	23 (95.8)	21 (87.5)	22 (91.7)	22 (95.7)
Mean number of implantations	13.5	14.2	14.3	14.0
Mean number of Corpora Lutea	16.9	16.7	17.3	16.5
Number of viable litters examined	22	21	22	22
Viable fetuses per group	270	284	302	285
Mean no. viable fetuses per dam	12.3	13.5	13.7	13.0
Mean no. early resorptions per dam	1.1	0.7	0.5	1.0
Mean no. late resorptions per dam	0	0	0	0.1
Mean no. total resorptios per dam	1.1	0.7	0.6	1.1
% post-implantation loss	8.8	4.7	4.1	7.8
Fetal sex ratio (% males)	51.9	49.3	48.3	46.0
Mean fetal body weight (g)	3.5	3.5	3.5	3.5
External foetal findings				
Number of fetuses examined	270	284	302	285
Anasarca	0	0	0	1[b]
Cleft lip	0	0	**2[a]**	0
Cleft palate	0	0	**1[a]**	**2[b]**
Club foot	0	0	1[a]	0
Micromelia	0	0	1[a]	0
Visceral findings				
Number of fetuses examined	141	148	156	148
Renal papilla(e) short	32	27	40	**57****
Renal papilla(e) absent	4	4	8	**16****
Dilated ureter(s)	38	21	38	**63****
Protruding tongue	0	0	0	1[b]
Hydromelia	0	0	0	1[b]
Selected skeletal findings				
Number of fetuses examined	129	136	146	137
Lacrimal bone agenesis	0	0	1[a]	0
Rudimentary ribs	**0**	**1**	**4**	**53**
No. litters with fetuses with rudimentary ribs	**0/22**	**1/21**	**4/22***	**16/22****
Sternebrae not ossified	49	54	**83***	**99****

Excludes the skeletal malformation of rudimentary 13th thoracic ribs observed in 3 control fetuses, 1 low dose and 1 high dose fetus.

*Statistically different from control at P<0.05, ** P<0.01, [a] Same fetus cleft palate, cleft lip, lacrimal bone agenesis, micromelia, club foot , [b] Same fetus cleft palate, protruding tongue, hydromelia, anasarca

Conclusions

The NOAEL for maternal toxicity was 30 mg/kg bw/day, based on reduced body weight gain and reduced food consumption at 90 and 360/300 mg/kg bw/day at the beginning of the treatment period. For fetal effects, a NOAEL of 30 mg/kg bw/day was established based on one cleft palate observed at 90 and two cleft palates at 360/300 mg/kg bw/day (all in different litters, 1/22 and 2/22 litters, respectively). Moreover, an increased incidence of skeletal variations (rudimentary ribs and non-ossified sternebrae) were observed at 90 mg/kg bw/day and 360/300 mg/kg bw/day, and increased incidence of visceral variations (short and absent renal papilla(e) and dilated ureters) at 360/300 mg/kg bw/day.

In this study, the incidence of cleft palate in the intermediate dose group was 1/302 (0.33%) and in the high dose group 2/285 (0.70%). Cleft palate is a rare malformation in CD rats. According to data submitted by the registrant the incidence of cleft palate was in the performing laboratory 0/5431 during 1983-1985, whereas in other laboratories the incidence was 4/25522, (0.016%) in 1983-1986. At intermediate dose maternal toxicity was moderate exhibited by transient decreases in body weight gain and food consumption during the first days of dosing. Although maternal toxicity was marked in high dose dams, there was no lethality and no effect on corrected body weight gain or on any of the reproductive or fetal parameters examined. Thus, although cleft palates were observed at maternally toxic doses, treatment-related effect cannot be excluded.

To clarify the equivocal cause of cleft palates, **a supplementary developmental toxicity study was carried out (DAR IIA 5.6.2/02)**. The design of the study was based on test guideline OECD 414, 1981 but with only one dose level, enhanced group size, and limited fetal examination.

A total of 400 sexually mature virgin female Crl:COBS CD (SD)BR rats were mated with 199 sexually mature males of the same strain. 189 sperm positive females were dosed by oral gavage with 300 mg/kg bw/day propiconazole (purity 88%) on gestation days 6-15. 178 sperm positive control females received an equivalent volume of the vehicle, 3% corn starch containing 0.5% Tween 80. All suspensions were found to be within ± 4% of the nominal concentration. The dams were observed twice daily for mortality and daily for changes in appearance and behaviour. Body weights were recorded on gestational days 0, 6, 8, 12, 16, and 20. Food consumptions were recorded for weekly period from day 0 to day 6, and daily thereafter through gestation. The dams were necropsied on day 20 of presumed gestation. The uteri were weighed and corpora lutea, live fetuses, dead fetuses and intrauterine resorption sites were counted. The fetuses were sexed and numbered in order of their positions in the uterus. Viable fetuses were weighed and examined for external abnormalities including careful check of the palate. The dams were examined for gross pathology and selected tissues were examined.

Results

Dams in the treated group showed significant increase in ataxia, coma, lethargy, prostration, respiratory difficulties and salivation. In addition ptosis, lacrimation and pale color were observed in treated dams (Table 43). Two unscheduled deaths in the treated group were attributed to test article toxicity. One additional dam from the treated group delivered prematurely and one died due to an intubation error. Food consumption and body weight gains of treated dams were significantly reduced during the treatment period of days 6-16 of gestation, but recovered to control levels following the cessation of dosing (Table 45). The absolute body weights of treated dams were significantly lower (3-4%) when compared to controls from day 8 of gestation onwards and were still significantly lower

than controls at terminal sacrifice on day 20. The corrected body weight gain of treated dams was significantly lower than controls (days 0-20, Table 44). There were no significant gross pathological findings.

Table 43: Summary of selected clinical observations (based on the number of animals displaying the observation on at least one occasion after the initiation of treatment)

Observation	Dose level of propiconazole	
	0 mg/kg bw/day	300 mg/kg bw/day
Ataxia	0/178	79/189**
Comatose	0/178	17/189**
Lethargy	0/178	83/189**
Prostration	0/178	5/189*
Respiration audible	0/178	7/189**
Respiration laboured	0/178	20/189**
Salivation	0/178	37/189**

*Statistically different from control at P<0.05, **Statistically different from control at P<0.01

Table 44: Maternal body weight gain (g) (% difference from control)

Days	Dose level of propiconazole	
	0 mg/kg bw/day	300 mg/kg bw/day
0-6	28.25	30.77
6-8	9.27	-2.74*
8-12	22.10	16.69* (24% ↓)
12-16	29.52	26.91* (9%↓)
16-20	58.27	62.99* (8%↑)
0-20	147.41	134.97* (8%↓)
0-20#	69.82	57.75* (17%↓)

*Statistically different from control at P<0.05, Days 0-20# = Body weight gain excluding gravid uterus weight

Table 45: Maternal food consumption (g/rat) - selected days

Days	Dose level of propiconazole	
	0 mg/kg bw/day	300 mg/kg bw/day
0-6	145.28	146.16
6-7	25.87	19.66* (24%↓)
7-8	25.24	15.91* (37%↓)
8-9	25.35	15.13* (40%↓)
9-10	25.66	18.41* (28%↓)
10-11	26.32	21.39* (19%↓)
15-16	29.19	25.92* (11%↓)
16-17	30.01	29.15
19-20	28.06	29.13

*Statistically different from control at P<0.05

The number of viable fetuses and fetal weights were significantly reduced in the treated group (Table 46). There was also slight, not statistically significant, increase in post-implantation loss in the treated dams. External examinations showed one case of agnathia, two cases of filamental tail and one case of multiple malformations in the control fetuses, and two cases of cleft palate in the treated fetuses (Table 46). The two cases of cleft palates occurred in different litters.

Table 46: Summary of reproductive and foetal findings

	Dose level of propiconazole	
Reproductive parameters	**0 mg/kg bw/day**	**300 mg/kg bw/day**
Number of pregnant females/No. placed on study	155/178	161/189
Mean number of. implantations	14.5	14.2
Mean number of Corpora Lutea	16.9	17.0
Number of litters examined	155	158[a]
Number of viable fetuses per group	2122	2064
Mean number of viable fetuses/dam	13.7	**13.1***
Number of early resorptions	0.8	1.0
Number of late resorptions	0.1	0.1
Total number of resorptions	0.81	1.15
% post-implantation loss	5.8	8.6
Fetal sex ratio (% males)	49	50
Fetal weight - males (g)	3.569	**3.403****
Fetal weight - females (g)	3.387	**3.232****
Malformations / number of fetuses examined		
Spina bifida, gastrochisis, exencephaly, protruding tongue	1[b]/2122	0/2064
Agnathia	1/2122	0/2064
Filament tail	2/2122	0/2064
Cleft palate	0/2122	**2/2064**[c]

*Statistically different from control at P<0.05, **P < 0.001, [a] Three dams died before termination of the study

[b] Same fetus spina bifida, gastrochisis, exencephaly, protruding tongue, [c] Fetuses from different litters

Conclusions

Oral administration of propiconazole at dose level of 300 mg/kg bw/day caused severe maternal toxicity manifested by signs of lethargy, coma, ataxia, respiratory difficulties, prostration, salivation and premature death of two dams. Fetal and reproduction toxicity (decreased number of viable fetuses, decreased fetal weight, and slight increase in post-implantation loss) was also observed at the tested dose level of 300 mg/kg bw/day. In the treated group, cleft palate was observed in 2/2064 fetuses (incidence 0.097%) from 2/158 litters. According to historical control data submitted by the registrant cleft palate occurred sporadically during 1983 to 1985 in this rat strain at an incidence ranging from 0% (0/5431, this laboratory) to 0.016% (4/25522, other laboratories).

In a developmental toxicity study by Raab (1986) propiconazole (purity 88%) was administered to three groups of 19 artificially inseminated New Zealand White female rabbits at dose levels of 100, 250 or 400 mg/kg bw/day. The test article was administered once daily by gastric intubation as a suspension in 3% cornstarch containing 0.5% Tween 80 from day 7 to day 19 of gestation at a volume of 5.0 ml/kg/day. Control animals received an equivalent volume of vehicle. All suspensions were found to be within ± 6% of the nominal concentrations. The does were observed daily for changes in appearance and behaviour, and mortality was checked twice daily. Body weights were recorded on days 0, 7, 10, 14, 20, 24, and 29 of gestation. Food consumption was measured daily during days 5 to 29 of gestation. Does were necropsied on day 29 of presumed gestation. The ovaries were examined and corpora lutea counted. The uteri were weighed and fetuses and intrauterine resorption sites were counted. Implantations were numbered in order of their positions in the uterus. Viable fetuses were weighed and examined for gross and visceral abnormalities and all fetuses were subjected to skeletal examination. The does were examined for gross pathological changes.

Results

One unscheduled death occurred in both the low (cause unknown) and intermediate dose groups (intubation error). In addition, one control doe, one doe from the intermediate group and five high dose dams were sacrificed prior to schedule because of early delivery or abortion. High dose group showed significantly increased incidence in stool variations (18/19 in the high dose group compared to 11/19 in controls), which was considered to be treatment related. Food consumption was significantly reduced during the whole dosing period in the intermediate and high dose groups (Table 47). After the cessation of treatment until sacrifice, food intake increased above control values in both groups. Body weight gain was slightly reduced in the intermediate and significantly reduced in the high dose group during the dosing period (up to 89% reduction compared to controls), and recovered thereafter (Table 48). The absolute body weights were significantly lower in the high dose group on day 20. On day 29, there were no statistically significant differences between the corrected body weights (minus uterus placentas and fetuses) of all groups. There were no abnormal necropsy findings.

Table 47: Maternal food consumption (g) - selected days

Days	Dose level of propiconazole (mg/kg bw/day)			
	0	100	250	400
5-6	193 (13)	189 (17)	191 (15)	198 (10)
7-8	183 (11)	162 (17)	136* (14) 26%↓	79* (12) 57%↓
11-12	174 (13)	159 (16)	133* (15) 24%↓	86* (12) 51%↓
17-18	173 (11)	171 (31)	109* (14) 37%↓	93* (11) 34%↓
20-21	175 (11)	156 (16)	141 (15)	149 (8)
23-24	104 (14)	123 (16)	131 (15)	150* (12) 44%↑
26-27	90 (12)	107 (15)	104 (14)	151* (11)
28-29	110 (13)	112 (15)	100 (12)	136 (11)

( ) = number of animals used in mean, *Statistically different from control at P<0.05

Table 48: Maternal body weight gain (kg)

Days	Dose level of propiconazole (mg/kg bw/day)			
	0	100	250	400
0-7	0.226 (14)	0.212 (17)	0.173 (15)	0.176 (12)
7-10	0.018 (14)	0.005 (17)	-0.047* (15)	-0.111* (12)
10-14	0.076 (14)	0.076 (17)	0.051 (15)	0.008* (12) 89%↓
14-20	0.144 (12)	0.134 (16)	0.111 (14)	0.063* (12) 56%↓
20-24	0.046 (13)	0.094 (17)	0.118* (15) 157%↑	0.159* (11) 246%↑
24-29	0.069 (14)	0.077 (17)	0.057 (15)	0.144 (12)
0-29	0.018 (14)	0.055 (17)	-0.130 (15)	-0.097 (12)

( ) = number of animals used in mean, * P<0.05, Days 0-29 = body weight gain excluding gravid uterus weight

No statistically significant differences were observed in the number of corpora lutea, number of implantation sites and number of viable or dead fetuses (Table 49). Incidences of resorptions and abortions or early deliveries were significantly increased among does of the high dose group. In the case of one high dose dam, the whole litter (ten pups) was resorbed early and there were no live pups at termination (the effect does not reach statistical significance if this dam is omitted from analysis). Five high dose dams were sacrificed prior to schedule because of early delivery (one doe) or abortion (four does, Table 49). In addition, one doe from the intermediate group aborted and one doe from the control group delivered early. The impact of maternal toxicity in relation to the abortion/premature delivery for high dose dams is not discussed in the report. The reported individual body weight data reveal that one dam loosed weight during the first 20 days of gestation prior to abortion on day 22. The data revealed no other signs of exceptionally severe toxicity in dams that aborted or delivered early.

Fetal weights were not affected by the treatment. Only one fetus had malformations; fetus from the intermediate dose group had a cleft lip, umbilical hernia and hydronephrosis with hydroureter. Five fetuses had visceral variations: one control fetus (red area in left lung), two intermediate dose group fetuses (thick aorta and small gallbladder, other fetus had ovarian cysts) and two high dose group fetuses (the same litter) had coagulated blood above bladder. Since majority of the gross and visceral observations were limited to single intermediate-dose group fetus, without any dose-response, there

were considered to be spontaneous in nature. Various skeletal variations were observed across all groups. Of these the incidence of fully formed 13th ribs, was significantly increased among the high dose group fetuses (Table 49).

Table 49: Summary of reproductive and foetal findings (see also text above)

	Dose level of propiconazole (mg/kg bw/day)			
Reproductive parameters	**0**	**100**	**250**	**400**
Number of pregnant does/ no. inseminated	15/19	18/19	17/19	18/19
Found dead/sacrificed	0	1	1	0
Aborted/Delivered early	1[a]	0	1[b]	**5*[c]**
Viable litters examined	14	17	15	12[d]
Mean no. Corpora Lutea	11.6	12.6	13.3	13.5
Mean no. implantations	8.4	9.4	10.0	9.2
Early resorptions: per group (mean per dam)	2 (0.1)	6 (0.4)	6 (0.4)	**17**[e] (1.3)
Late resorptions per group (mean per dam)	8 (0.6)	6 (0.4)	5 (0.3)	10 (0.8)
Total resorptions per group (mean per dam)	10 (0.7)	12 (0.7)	11 (0.7)	**27* (2.1*)**
Viable fetuses per group (mean per dam)	101 (7.2)	146 (8.6)	130 (8.7)	93 (7.2)
Dead fetuses per group (mean per dam)	6 (0.4)	1 (0.1)	9 (0.6)	0 (0)
Fetal sex ratio (% males)	55	49	50	41
Fetal weight (g), males/females	43.0/44.2	44.4/43.1	42.8/41.1	42.8/43.2
Gross malformations				
Number of fetuses examined[f]	101	146	130	93
Cleft lip	0	0	1[g]	0
Umbilical hernia	0	0	1[g]	0
Visceral malformations				
Hydronephrosis with hydroureter	0	0	1[g]	0
Selected skeletal variations				
Fully formed (13th) ribs	35	63	58	**63***
Rudimentary ribs	32	20	22	11
Floating rudimentary ribs	0	5	4	2
Wavy ribs	0	0	0	1

*Statistically different from control at P<0.05, [a] delivery on day 29, [b] abortion on day 21

[c] 3 dams aborted on day 26, one aborted on day 22 and one delivered on day 29

[d] one doe was pregnant but had no vible fetuses at terminal sacrifice, [e] one doe resorbed the whole litter, 10 pups.

[f] no. of fetuses examined for gross malformations, visceral malformations and skeletal variations

[g] same fetus cleft lip, umbilical hernia, hydoronephrosis with hydroureter, thick aorta and small gallbladder

Conclusions

Oral administration of propiconazole to pregnant female rabbits suggests NOAEL of 100 mg/kg bw/day for maternal toxicity, based on reduced body weight gain and reduced food consumption at higher doses. For fetal effects and reproduction, NOAEL of 250 mg/kg bw/day was established based

on resorptions, abortions or early deliveries, and because of the increased incidence of fully formed 13th ribs at 400 mg/kg bw/day.

4.10.2.2 Human information

No information available.

4.10.3 Other relevant information

Propiconazole, as well as other conazoles, induces its antifungal functions *via* binding to the heme group of CYP51 (also known as sterol 14α-demethylase) causing inhibition of its function. In addition, propiconazole may inhibit functions of other CYP enzymes, such as CYP19 (also known as aromatase), and thus induce imbalance in the steroidogenesis and exert endocrine disrupting effects (Zarn et al. 2003). A variety of studies on potential endocrine disrupting effects of propiconazole have been published in open scientific literature and conducted by request of other authorities. These studies are briefly reviewed here with regard to *in vivo* effects on fertility and development.

The effect of propiconazole exposure on reproduction and maturation of offspring has been studied by Goetz *et al* (2007). Wistar Han rats were fed diets containing 0, 100 ppm, 500 or 2500 ppm propiconazole from gestation day 6, through gestation and lactation. On postnatal day (PND) 8, litters were weighed and culled to eight pups per dam, retaining males preferentially. The F_1 generation continued on the same treated diets from weaning (PND23) until PND120. There were no treatment-related effects on the fertility and fecundity parameters (insemination and fertility indexes of PND 78 or older treated F1 males, total number of implantation sites, number of early and late dead embryos, fetus viabilities). All successful pregnancies produced normal healthy litters with little to no post implantation loss. The anogenital distance on PND0 was significantly increased following exposure to 2500 ppm (144-174 mg/kg bw/day) and trend analysis showed a significant increasing trend. There was no treatment-related effect on the day of preputial separation. Testes weights were increased on PND50 at 500 ppm (53 mg/kg bw/day) and on PND22 at 2500 ppm (205-413 mg/kg bw/day). There were no treatment-related histological findings in the pituitary, thyroid, testis, epididymis or ventral prostate. There were no treatment-related effects on sperm morphology or motility. Serum testosterone levels were increased at PND92 at 500 ppm and 2500 ppm. There was no effect on serum levels of oestradiol, LH, total T3, total T4, and TSH levels. It was proposed that altered steroid homeostasis caused the observed increases in serum testosterone, anogenital distance and testes weights.

In another study, oral administration of propiconazole 50 mg/kg bw/day to pregnant Wistar rats from gestational day 7 to 21 caused no effects on maternal body weight, numbers of implantations, number of live foetuses, rate of post-implantation loss, sex ratio, or male or female anogenital distance (Taxvig *et al* 2008). Male and female foetal weights were increased (statistically significantly for males). In propiconazole treated dams, there was a statistically significant increase in serum 17α-hydroxyprogesterone and small but not significant increase in testosterone but no effect on progesterone or oestradiol levels. The authors concluded that the increased foetal weight may be related to the up-regulated levels of testosterone observed in the dams.

Taxvig *et al* (2008) also addressed the potential of propiconazole to affect male fertility through anti-androgenic effects using Hershberger assay. Administration of 50, 100 or 150 mg/kg bw/day propiconazole to castrated testosterone-treated Wistar rats had no effect on reproductive organ weights (testes, ventral prostate, combined seminal vesicles and coagulating glands, bulbourethal glands). The serum concentration of follicle stimulating hormone (FSH) was significantly increased at 150 mg/kg bw/day. No other differences in hormone levels (LH, FSH, T_4) were observed. It was

concluded that propiconazole had no antiandrogenic effects. Moreover, in the study by Tully *et al* (2006) groups of six 60 day old male Sprague Dawley rats were gavage dosed for 14 days with 10, 75, or 150 mg/kg bw/day propiconazole. This study revealed no effects on testes weights or histology, sperm morphology or motility or any of the serum hormones measured (testosterone, LH, FSH, oestradiol). In contrast, when Wistar male rats were treated with 4 mg/kg bw/day propiconazole from PND 50 to 120, significant increase in abnormal sperm tail morphology, increased seminal vesicle and vas deferens weight, and decreased serum estradiol levels were observed (Costa *et al* 2015). There were no effects on other sperm parameters or morphometric parameters of testis. The higher propiconazole dose used in the study, 20 mg/kg bw/day, impaired sexual behavior of males (increased latency after first ejaculation), but no other treatment-related effects were observed.

Effect of propiconazole on female rat reproductive development was addressed in the study by Rockett *et al* (2006). Wistar han rats were fed diet containing 0, 100, 500 or 2500 ppm propiconazole from gestation day 6 through gestation, parturition, and lactation. Litters were culled to eight pups on PND8, and the pups were weaned on PND22. Female weanlings were continued on the same diet as their mothers until termination on PND98. Body weight at PND0, anogenital distance on PND0 and timing of vaginal opening were unaffected by treatment. Decreased food intake, increased liver weight and histopathological signs of liver toxicity were observed at 2500 ppm. Oestrous cyclicity was disrupted on first two weeks after vaginal opening at 500 ppm (increase of abnormal cycles) and 2500 ppm (increase of extended cycles) propiconazole but later normalized (assesments on weeks 5-6 and 9-10). There were no treatment-related histological findings in ovaries. It was concluded that exposure to high concentrations of propiconazole adversely impacted the reproductive development of the female rat. The effects appeared to be either short term or reversible and probably reflected either the declining relative dose levels and/or the changing physiology of the rats as they matured. The observed changes were considered consistent with either an antioestrogenic or androgenic mechanism (the former is suspected but not verified).

In conclusion, the above reviewed *in vivo* studies revealed no major effects of propiconazole on fertility or reproduction. The reported increases in serum testosterone, pup weights, testes weights, and in anogenital distance are consistent with the proposed ED MoA of propiconazole (interference with steroidogenesis). In addition, abnormal sperm tail morphology after exposure to low dose and impaired sexual behavior and disrupted oestrus cycle following mid and high doses of propiconazole were reported (Rockett *et al* 2006, Costa *et al* 2015). However, taken into account the partial controversy of the findings between studies, and the obtained negative results on fertility and reproduction, their biological significance is presently obscure.

4.10.4 Summary and discussion of reproductive toxicity

Propiconazole did not affect fertility, mating or gestation **in a rat two generation reproduction study.** Signs of liver toxicity (hepatocyte hypertrophy and vacuolisation) associated with reduced body weights and food consumption were observed in parental animals at intermediate (49 mg/kg bw/day in males, 44 mg/kg bw/day in females) and high doses (215 mg/kg bw/day in males, 243 mg/kg bw/day in females). Hepatocyte hypertrophy was also observed in progeny of high dose dams. During first generation the high dose caused significantly reduced pup weights over lactation period. During second generation also reductions in litter size, number of viable pups delivered, pup survival over lactation period and increased number of runt pups were observed at the high dose. Taken into account the histopathological liver findings in both parental animals and the progeny at this dose as well as findings of the repeated dose toxicity studies, it seems plausible that these effects are secondary consequence of systemic toxicity rather than direct effect of propiconazole on reproduction. Therefore these effects are not considered to warrant classification. Testes weights of

male offspring (second litter of both generations) exposed to high dose (215 mg/kg bw/day) were significantly reduced. Since histopathological examinations were only performed to first litters of the generations (with negative findings), this finding was not confirmed by microscopy. Hence, oestrus cycle, sperm parameters and parameters of sexual maturation of the offspring were not determined in this study. Repeated dose studies and chronic toxicity studies either did not address testes weights and histopathology, or examinations did not reveal any treatment-related findings (sections 4.7. and 4.9 of this report). Thus, the biological significance of this effect remains questionable. The studies published in open scientific literature have not revealed any major effects of propiconazole on fertility (see section 4.10.3). Some effects consistent with the proposed ED MoA of propiconazole (interference with steroidogenesis) have been reported (e.g. increased testes weigths and anogenital distance, reversible disruption of oestrus cycle). However, taken into account the partial controversy of the findings between studies, and the obtained negative results on fertility and reproduction, their biological significance is presently obscure. Overall, it is considered that the data presently available on propiconazole does not warrant classification for fertility effects.

The potential of propiconazole to induce **prenatal developmental toxicity** was investigated in two rat studies and one rabbit study**.** In rat oral developmental toxicity study cleft palates occurred in 1/302 (0.33%) pups at intermediate dose (90 mg/kg bw/day) and in 2/285 pups (incidence 0.7%) of different litters at the high dose (360/300 mg/kg bw). In a supplementary developmental toxicity study cleft palate was again observed, but with lower incidence (2/2064 fetuses from 2/158 litters, incidence 0.097%), suggesting reproducibility of this malformation upon propiconazole exposure. The study report states that cleft palate had not been seen previously in the performing laboratory (incidence 0/5431 during 1983-1985) and according to data submitted by the registrant the observed incidences are also above the historical control data of other laboratories during 1983-1986 (4/25522 , 0.016%).

Cleft palate is a malformation that can be induced by chemicals if the critical dose and timing of exposure are aligned. It has also been suggested that cleft palates could occur as a consequence of maternal toxicity. In both rat developmental toxicity studies marked maternal toxicity was evident at the high dose. Maternal toxicity was more severe in the supplementary study resulting death of two dams. In this study the number of viable fetuses and fetal weights were significantly reduced and there was also slight, not statistically significant, increase in post-implantation loss in the treated dams. However, the incidences of cleft palates were higher in the main study where one cleft palate occurred also at intermediate dose (90 mg/kg bw/day) associated with only moderate maternal toxicity. Increased incidence of cleft palates in rat has also been observed in response to exposure to other triazoles (e.g. cyproconazole and epoxiconazole). The mechanism is not known, but the teratogenicity of triazoles is hypothesized to be related to altered embryonic retinoid acid catabolism since abnormalities are confined to structures controlled by retinoid acid (Giavini and Menegola, 2010). There is no information showing that the mechanism is not relevant for humans. Therefore, although the incidences of cleft palates in rats were low in the two studies with propiconazole, and occurred with moderate to marked maternal toxicity, it cannot be ruled out that the effect is treatment-related and classification of propiconazole for developmental effects should be considered.

In rat, significantly increased incidence of skeletal variations (rudimentary ribs and non-ossified sternebrae) at intermediate (90mg/kg bw/day) and high doses (360/300 mg/kg bw/day), and increased incidence of urinary tract variations at high dose were also observed. These variations occurred in association with maternal toxicity, and thus they may represent a delay in growth and development secondary to maternal toxicity.

In a rabbit developmental toxicity study administration of 400 mg/kg bw/day (high dose) propiconazole caused marked maternal toxicity, increased incidences of resorptions, abortions and early deliveries in does and an increased incidence of fully formed 13th rib in fetuses. The increased

incidence of fully formed 13th rib is a variation and occurred only at high dose in association with marked maternal toxicity, and may therefore be a secondary consequence of maternal toxicity. However, it is not possible to conclude whether increased incidences of resorptions, abortions and early deliveries in rabbit were secondary to maternal toxicity. Thus, these effects cause additional concern for developmental toxicity of propiconazole.

4.10.5 Comparison with criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A, known human reproductive toxicant) or from animal data (Category 1B, presumed human reproductive toxicant).

There is no human data available on propiconazole therefore classification in Category 1A is not appropriate. The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development 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 other toxic effects. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such 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.

Cleft palate is a malformation. However, increased incidence of cleft palates was observed only in one species, in low incidences and in association with maternal toxicity. These factors reduce the concern for developmental hazard and therefore classification as Repr. 1B is not considered appropriate. Since it cannot be ruled out that the effect is treatment-related and also relevant for humans, therefore primarily based on low incidences of cleft palates in two rat studies, propiconazole is proposed to be classified as **Repr. 2; H361d** (Suspected of damaging the unborn child). No classification is proposed for adverse effects on fertility.

4.10.6 Conclusions on classification and labelling

CLP: Repr. 2; H361d

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

The DS proposed no classification of propiconazole for sexual function and fertility effects. It was based on a 2-generation reproduction study in rats showing negative results on mating, fertility, gestation, female and male fertility index and average of gestation length. The only effects on reproduction reported in this study were reduction in pup weights during the first generation and during the second generation reductions in litter size, number of viable pups delivered, pup survival and increased number of runt pups at the highest dose.

Developmental toxicity

The DS proposed classification of propiconazole for reproductive toxicity category 2 (H361d) on the basis of two different developmental studies in rat showing incidences of cleft palates higher than controls and historical control data but appearing always concurrently with maternal toxicity.

Comments received during public consultation

Three MSCAs supported the classification proposed by the DS.

One MSCA disagreed with the proposal of classification as category 2 and proposed category 1B considering that: i) the increased incidence of cleft palates in rats treated with propiconazole justifies classification for developmental toxicity; ii) there is no convincing evidence demonstrating that the sensitivity of humans is more similar to rabbits than rats; iii) disagreement about a reduction of the concern by high maternal toxicity; iii) the incidences of cleft palate were observed in the two foetuses occur in different litters; iv) it cannot be excluded that some additional cases may be masked by the slightly increased post implantation loss and reduced number of viable foetuses in the developmental rat study; and, v) this rare malformation is commonly observed with other "conazoles". The DS agreed with the comments and leaved the final decision in RAC.

One MSCA requested a classification on fertility on the basis of oestrus cycle and anogenital distance. The DS replied that there are a few findings in the open scientific literature which add concern for reproductive toxicity of propiconazole and that were discussed in the CLH report. However, the DS chose not to propose classification for fertility for propiconazole because of the following reasons: i) there were no significant effects on fertility, fecundity or reproduction parameters in a two-generation reproduction study or when assessed, in studies published in open scientific literature; ii) increase of anogenital distance in male pups and reversible disruption of oestrus cycle may rather contribute to classification for developmental toxicity (reproductive development) than for fertility; iii) although all these effects suggest for disturbed steroidogenesis they may be considered as individual findings because when assessed, effects on anogenital distance or oestrus cycle have not been observed in other studies.

One MSCA requested more information for establishing a read-across with other triazoles. The DS did not consider it necessary since this information is already documented in the respective RAC opinions.

One manufacturer/company diminished the relevance of the cleft palate cases on the basis of low incidence, maternal toxicity, absence of embryo lethality and lack of evidences about if propiconazole-induced effects would result in functional deficiency in foetuses. The company also submitted a position paper requesting no classification for propiconazole together with another published historical control data and a third rat developmental study showing no cases of cleft palate. DS disagreed with the proposal of no classification considering that the incidence of cleft palate were above the historical control of the performing laboratory and noted that the third new study contains certain deviations from the OECD TG 414 that do not allowed the DS to assess the acceptability of the study. In

addition, the DS is of the opinion that the negative findings of this study do not overrule the findings of other developmental toxicity studies.

Another MSCA highlighted that propiconazole is included in the Endocrine Disruptor Screening Program Tier 1 and therefore several other studies on endocrine properties are available and requested more discussion about the appropriate classification for developmental toxicity. The DS answered that endocrine disruption *per se* is not a hazard considered by the CLP Regulation. However, the disruption of endocrine receptors may form part of one or more MoA of a chemical considered by RAC under the Reprodutive toxicity hazard class.

An international NGO submitted a comment that classification in category 1 is needed on the basis of papers published in the open scientific literature on reproductive toxicity and on endocrine disruption.

Assessment and comparison with the classification criteria

Sexual function and fertility

In a two-generation reproduction study, propiconazole was administered in the diet (*ad libitum*) at concentrations 0, 100, 500 and 2500 ppm to groups of 15 male and 30 female Charles River CD rats. The main deviations to current OECD TG 416 were: oestrus cycle and sperm parameters were not determined, developmental landmarks of the offspring including parameters of sexual maturation were not evaluated, food consumption was only determined during pre-mating period, only brain, ovary and testes weights were determined.

Statistical analysis of reproductive data (mating, fertility, gestation, female fertility and male fertility index, average gestation length) revealed no significant reductions in these parameters. Delivery and population data (the mean numbers of pups delivered, delivered viable, stillborn and partially cannibalized at birth, the numbers of survived pups during the lactation period) obtained for the groups of dams exposed to propiconazole were comparable to the control dams during both the F_{1a} and F_{1b} litters. In the F_{2a} litter, the number of pups delivered, delivered viable and surviving to lactation day 4, were significantly reduced for the 2500 ppm dams (8.1 in the treated animals versus 12.0 in control group). In the F_{2b} litter, the pup survival indices at lactation days 7, 14, and 21 were significantly reduced for the 2500 ppm group dams (6.2, 5.7 and 5.7 in exposed animals versus 7.7, 7.7 and 7.7 in control group, respectively).
The histopathological analysis revealed statistically significant increases in liver hypertrophy of males and females of all generations at the highest dose and in F_0 and F_1 males and females at 500 ppm (table below). F_0 and F_1 males and females showed liver vacuolation at 2500 ppm, while the incidence of this vacuolation was significant at 500 ppm only in F_1 males.

Table: Histopathological changes in liver of parental animals and progeny. Bolded figures highlight the statistically significant differences regarding the animals dieted with 0 ppm propiconazole.

	Dietary concentration of propiconazole (ppm)							
	Males				Females			
	0	100	500	2500	0	100	500	2500
Hypertrophy								
F_0	7/15	3/15	**13/15**	**14/15**	4/30	3/29	6/30	**29/30**
F_1	0/15	1/15	**5/15**	**15/15**	0/30	2/30	**15/30**	**29/30**
F_{1b}	2/10	1/10	2/10	**10/10**	1/10	1/10	2/10	**8/10**
F_{2b}	0/10	0/10	2/10	**10/10**	0/10	0/10	1/10	**9/10**
Vacuolation								

F_0	0/15	2/15	3/15	**14/15**	1/30	1/29	1/30	1/30
F_1	2/15	5/15	**8/15**	**11/15**	2/30	4/30	7/30	**10/30**
F_{1b}	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
F_{2b}	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10

Absolute brain and testes (including epididymides) weights of the 2500 ppm group of F_{1a} males were significantly reduced in comparison to controls. Absolute testes weights and testes to brain weight ratios of 2500 ppm F_{2a} males were also significantly reduced compared to controls. The testes findings were not confirmed by microscopy, since histopathological examinations were only performed on F_{1b} and F_{2b} litters. A significant increase in the brain to body weight ratio was noted in the 2500 ppm F_{2b} males and a significant reduction in the brain weight was noted in the 2500 ppm F_{2b} females. RAC notes that the incidence of the above mentioned effects was not stated in the CLH report.

Development

Two-generation reproduction study in rats

In a two-generation reproduction study (see also 'Sexual function and fertility') propiconazole was administered in the diet (*ad libitum*) at concentrations 0, 100, 500 and 2500 ppm to groups of 15 male and 30 female Charles River CD rats.

In F_{1a} and F_{2a} litters, progeny of dams exposed to 2500 ppm had significantly lower body weights than controls on lactation days from 4, 7, 14 and 21 (table below). Body weights of 2500 ppm F_{1b} progeny were significantly lower than those of the control progeny on lactation days 14 and 21. Body weights of 2500 ppm F_{2b} progeny were significantly lower than the control progeny on lactation days from 0, 4, 7, 14 and 21. The body weight data obtained for the 100 and 500 ppm progeny revealed inconsistent statistically significant reductions and increases in comparison to controls with equivocal biological significance.

Table: Effect of propiconazole on body weight of pups of different litters.

		Mean pup body weight (g)			
		0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**
F_{1a}	0	6.1	6.0	6.1	6.2
	4	8.9	8.8	9.2	**8.1****
	7	14.6	**13.0****	15.2	**12.3****
	14	28.2	**25.4****	**26.9***	**21.9****
	21	48.0/46.1	**44.1/42.5****	47.7/44.9	**35.8/34.7****
F_{1b}	0	6.1	6.0	**6.4****	6.1
	4	8.8	9.2	**9.5****	8.6
	7	13.4	14.5	**14.5***	13.3
	14	25.7	**27.7***	**28.0****	**22.9****
	21	44.3/40.1	46.1/43.5	47.7/43.5	**35.6/32.5****
F_{2a}	0	5.4	5.3	**5.7***	5.4
	4	8.5	**7.9****	8.3	**7.5****
	7	13.7	13.3	13.0	**10.8****
	14	25.6	25.4	25.2	**20.0****
	21	438./41.1	42.5/40.4	425./39.8	**31.5/30.2****
F_{2b}	0	5.8	**5.4****	**5.5****	**5.4****
	4	8.8	8.6	8.5	**7.3****
	7	14.6	14.7	13.8	**10.9****
	14	29.4	28.7	**26.8****	**21.9****
	21	48.8/46.2	48.6/46.6	**45.1/42.3****	**36.7/33.3****

Day 21, pup body weights are males/females ** statistically significant difference $p<0.01$, * $p<0.05$

Examination of the external structural development of the progeny did not reveal any statistically significant differences. Two 100 ppm F_{1a} anomalous pups (anurous and club limbs; cleft lip), one 2500 ppm F_{1a} anomalous pup (a partially opened left eyelid and a left eye which was smaller than normal), two 2500 ppm F_{1b} anomalous pups (a domed forehead [the brain was dilated] and eyes which appeared smaller than normal; a left eye which was enlarged with an opacity) and one 2500 ppm F_{1b} pup with an unopened eyelid (the eye was however not missing, and apparently of normal size) were obtained from dams treated with propiconazole. One stillborn 500 ppm F_{2a} pup exhibited agnathia and possible exencephaly (dam cannibalized top of head). One 100 ppm F_{2b} pup displayed clubbed limbs, shortened torso and a shortened tail. One 500 ppm F_{2b} pup kept its left eyelid closed (the left eye was shrunken in size). Number of runt pups was significantly increased in the 2500 ppm F_{2b} progeny.

Rat developmental toxicity study (DAR IIA 5.6.2/01)

The table in the STOT RE section summarises the maternal toxicity found in this study. Severe clinical signs were seen only at the highest dose of 360 mg/kg bw/d, which caused a reduction to 300 mg/kg bw/d. Despite maternal toxicity, all reproduction parameters remained similar in all groups (see table below). There were no significant differences in foetal weights between treated groups and controls. External and visceral examinations revealed one foetus in intermediate group to have cleft lip and cleft palate, micromelia and a club foot, and other foetus from different litter in the intermediate group to have cleft lip. In the high dose group one foetus had cleft palate, and another foetus from different litter had anasarca, cleft palate, hydromelia and protruding tongue. Significant increases in the incidences of short and absent renal papilla(e) and dilated ureters were detected in foetuses of the high dose group. Significant increases in the incidences of rudimentary ribs and non-ossified sternebrae were observed in foetuses of the high and intermediate dose groups.

Table: Summary of reproductive parameters and foetal findings.

	Dietary propiconazole (mg/kg bw/d)			
	0	**30**	**90**	**360/300**
Number of pregnant females (% of mated)	23 (95.8)	21 (87.5)	22 (91.7)	22 (95.7)
Mean number of implantations	13.5	14.2	14.3	14.0
Mean number of Corpora Lutea	16.9	16.7	17.3	16.5
Number of viable litters examined	22	21	22	22
Viable foetuses per group	270	284	302	285
Mean no. viable foetuses per dam	12.3	13.5	13.7	13.0
Mean no. early resorptions per dam	1.1	0.7	0.5	1.0
Mean no. late resorptions per dam	0	0	0	0.1
Mean no. total resorptions per dam	1.1	0.7	0.6	1.1
% post-implantation loss	8.8	4.7	4.1	7.8
Foetal sex ratio (% males)	51.9	49.3	48.3	46.0
Mean foetal body weight (g)	3.5	3.5	3.5	3.5
External foetal findings				
Number of foetuses examined	270	284	302	285
Anasarca	0	0	0	1[b]
Cleft lip	0	0	**2[a]**	0
Cleft palate	0	0	**1[a]**	**2[b]**
Club foot	0	0	1[a]	0
Micromelia	0	0	1[a]	0
Visceral findings				
Number of foetuses examined	141	148	156	148
Renal papilla(e) short	32	27	40	**57****

Renal papilla(e) absent	4	4	8	**16****
Dilated ureter(s)	38	21	38	**63****
Protruding tongue	0	0	0	1[b]
Hydromelia	0	0	0	1[b]
Selected skeletal findings				
Number of foetuses examined	129	136	146	137
Lacrimal bone agenesis	0	0	1[a]	0
Rudimentary ribs	**0**	**1**	**4**	**53**
No. litters with foetuses with rudimentary ribs	**0/22**	**1/21**	**4/22***	**16/22****
Sternebrae not ossified	49	54	**83***	**99****

Excludes the skeletal malformation of rudimentary 13th thoracic ribs observed in 3 control foetuses, 1 low dose and 1 high dose foetus.
*Statistically different from control at p<0.05, ** p<0.01, [a] Same foetus cleft palate, cleft lip, lacrimal bone agenesis, micromelia, club foot , [b] Same foetus cleft palate, protruding tongue, hydromelia, anasarca.

In conclusion, a NOAEL of 30 mg/kg bw/d for foetal effect was established based on one cleft palate observed at 90 mg/kg bw/d and two cleft palates at 360/300 mg/kg bw/d (all in different litters). Moreover, an increased incidence of skeletal variations (rudimentary ribs and non-ossified sternebrae) were observed at 90 mg/kg bw/d and 360/300 mg/kg bw/d, and increased incidence of visceral variations (short and absent renal papilla(e) and dilated ureters) at 360/300 mg/kg bw/d.

In this study, the incidence of cleft palate in the intermediate dose group was 1/302 (0.33%) and in the high dose group 2/285 (0.70%). Cleft palate is a rare malformation in CD rats. According to data submitted by the registrant, the incidence of cleft palate in the performing laboratory was 0/5431 during 1983-1985, whereas in other laboratories the incidence was 4/25522, (0.016%) in 1983-1986. At the intermediate dose, maternal toxicity was moderately exhibited by transient decreases in body weight gain and food consumption during the first days of dosing. Although maternal toxicity was marked in high dose dams, there was no lethality and no effect on corrected body weight gain or on any of the reproductive or foetal parameters examined. Thus, although cleft palates were observed at maternally toxic doses, treatment-related effect cannot be excluded.

Supplementary developmental toxicity in rat (DAR IIA 5.6.2/02)

The table in the STOT RE section summarises the main maternal effects of 300 mg propiconazole/kg bw/d that include clinical sings with two dead and reductions (17%) in maternal body weight gain. The table below summarises the results of the study regarding reproductive parameters and foetal findings.

Table: Summary of reproductive parameters and foetal findings.

	Dietary propiconazole (mg/kg bw/d)	
	0	**300**
Number of pregnant females/No. placed on study	155/178	131/189
Mean number of. implantations	14.5	14.2
Mean number of Corpora Lutea	16.9	17.0
Number of litters examined	155	158[a]
Number of viable foetuses per group	2122	2064
Mean number of viable foetuses/dam	13.7	**13.1***
Number of early resorptions	0.8	1.0
Number of late resorptions	0.1	0.1
Total number of resorptions	0.81	1.15
% post-implantation loss	5.8	8.6
Foetal sex ratio (% males)	49	50
Foetal weight - males (g)	3.569	**3.403****

Foetal weight - females (g)	3.387	**3.232****
Malformations / number of foetuses examined		
Spina bifida, gastrochisis, exencephaly, protruding tongue	1[b]/2122	0/2064
Agnathia	1/2122	0/2064
Filament tail	2/2122	0/2064
Cleft palate	0/2122	**2/2064[c]**

*Statistically different from control at p<0.05, **p < 0.001, [a] Three dams died before termination of the study
[b] Same foetus spina bifida, gastrochisis, exencephaly, protruding tongue, [c] Foetuses from different litters

In conclusion, oral administration of propiconazole at dose level of 300 mg/kg bw/d caused severe maternal toxicity (including premature death of two dams). Foetal and reproduction toxicity (decreased number of viable foetuses, decreased foetal weight, and slight increase in post-implantation loss) was also observed at the tested dose level of 300 mg/kg bw/d. In the treated group, **cleft palate was observed in 2/2064 foetuses (incidence 0.097%) from 2/158 litters**. According to historical control data submitted by the registrant cleft palate occurred sporadically during 1983 to 1985 in this rat strain at an incidence ranging from 0% (0/5431, this laboratory) to 0.016% (4/25522, other laboratories).

Supplementary developmental toxicity in rat

This study was submitted by Industry during the Public Consultation. It was performed with the objective to assess possible adverse effects of propiconazole on embryonic and/or foetal development. The study was conducted prior to Regulatory Test Guidelines and GLP, although following the principles of OECD TG 414. The major reported deviation was the dosing window, which was conducted from GD 6 to 15.

The dosage regime was 30, 100 and 300 mg/kg bw/d during days 6-15 of pregnancy. Dams of the highest dose reacted to the treatment by a marked reduction in body weight gain and food consumption and with mortality in three of the 25 dosed females (two on day 19 and one on day 20).

The gross examination of the foetuses did not reveal any treatment related malformation in any of the experimental groups. One case of hydrocephaly was reported in the 100 mg/kg bw/d group, which was within the historical control range. No pathological changes of the viscera were reported in any of the foetuses.

In both the 100 mg/kg bw/d and control groups, one case of irregularly sternum was reported. There was an increase in the number of un-ossified phalangeal nuclei of the fore and hind limbs in the 300 mg/kg bw/d group.

Developmental toxicity study in rabbit (DAR IIA 5.6.2/03)

On day 29, there were no statistically significant differences between the corrected body weights (minus uterus placentas and foetuses) of all groups. There were no abnormal necropsy findings.

The table below summarises the results of the study regarding reproductive parameters and foetal findings. No statistically significant differences were observed in the number of corpora lutea, number of implantation sites and number of viable or dead foetuses. Incidences of resorptions and abortions or early deliveries were significantly increased among dams of the high dose group. In the case of one high dose dam, the whole litter (ten pups) was resorbed

early and there were no live pups at termination (the effect does not reach statistical significance if this dam is omitted from analysis). Five high dose dams were sacrificed prior to schedule because of early delivery abortion. In addition, one doe from the intermediate group aborted and one from the control group delivered early.

Foetal weights were not affected by the treatment. Only one foetus had malformations; foetus from the intermediate dose group had a cleft lip, umbilical hernia and hydronephrosis with hydroureter. Five foetuses had visceral variations: one control foetus (red area in left lung), two intermediate dose group foetuses (thick aorta and small gallbladder, other foetus had ovarian cysts) and two high dose group foetuses (the same litter) had coagulated blood above bladder. Since majority of the gross and visceral observations were limited to single intermediate-dose group foetus, without any dose-response, there were considered to be spontaneous in nature. Various skeletal variations were observed across all groups. Of these the incidence of fully formed 13th ribs, was significantly increased among the high dose group foetuses.

Table: Summary of reproductive parameters and foetal findings.

	Dietary propiconazole (mg/kg bw/d)			
	0	**100**	**250**	**400**
Number of pregnant does/ no. inseminated	15/19	18/19	17/19	18/19
Found dead/sacrificed	0	1	1	0
Aborted/Delivered early	1[a]	0	1[b]	**5*[c]**
Viable litters examined	14	17	15	12[d]
Mean no. Corpora Lutea	11.6	12.6	13.3	13.5
Mean no. implantations	8.4	9.4	10.0	9.2
Early resorptions: per group (mean per dam)	2 (0.1)	6 (0.4)	6 (0.4)	**17[e] (1.3)**
Late resorptions per group (mean per dam)	8 (0.6)	6 (0.4)	5 (0.3)	10 (0.8)
Total resorptions per group (mean per dam)	10 (0.7)	12 (0.7)	11 (0.7)	**27*(2.1*)**
Viable foetuses per group (mean per dam)	101 (7.2)	146 (8.6)	130 (8.7)	93 (7.2)
Dead foetuses per group (mean per dam)	6 (0.4)	1 (0.1)	9 (0.6)	0 (0)
Foetal sex ratio (% males)	55	49	50	41
Foetal weight (g), males/females	43.0/44.2	44.4/43.1	42.8/41.1	42.8/43.2
Gross malformations				
Number of foetuses examined[f]	101	146	130	93
Cleft lip	0	0	1[g]	0
Umbilical hernia	0	0	1[g]	0
Visceral malformations				
Hydronephrosis with hydroureter	0	0	1[g]	0
Selected skeletal variations				
Fully formed (13th) ribs	35	63	58	**63***
Rudimentary ribs	32	20	22	11
Floating rudimentary ribs	0	5	4	2
Wavy ribs	0	0	0	1

*Statistically different from control at p<0.05,
a delivery on day 29,
b abortion on day 21
c 3 dams aborted on day 26, one aborted on day 22 and one delivered on day 29
d one doe was pregnant but had no viable foetuses at terminal sacrifice, e one doe resorbed the whole litter, 10 pups.
f no. of foetuses examined for gross malformations, visceral malformations and skeletal variations
g same foetus cleft lip, umbilical hernia, hydoronephrosis with hydroureter, thick aorta and small gallbladder

In conclusion, following oral administration of propiconazole to pregnant female rabbits, a NOAEL for foetal effects of 250 mg/kg bw/d was established based on resorptions, abortions or early deliveries, and because of the increased incidence of fully formed 13th ribs at 400 mg/kg bw/d.

Other relevant information

A variety of studies on potentially endocrine disrupting effects of propiconazole have been published in the open scientific literature.

The effect of propiconazole exposure on reproduction and maturation of offspring has been studied by Goetz *et al*. (2007). The anogenital distance was significantly increased following exposure to 2500 ppm (144-174 mg/kg bw/d). Testes weights were increased on post-natal day 50 at 500 ppm (53 mg/kg bw/d) and on post-natal day 22 at 2500 ppm (205-413 mg/kg bw/d). Serum testosterone levels were increased at post-natal day 92 at 500 ppm and 2500 ppm. It was proposed that altered steroid homeostasis caused the observed increases in serum testosterone, anogenital distance and testes weights.

In another study (Taxvig *et al*., 2008), oral administration of propiconazole 50 mg/kg bw/d to pregnant Wistar rats from GD 7 to 21 caused a statistically significant increase in serum 17α-hydroxyprogesterone and a small but not significant increase in testosterone and no effect on progesterone or oestradiol levels or on anogenital distance.

Taxvig *et al*. (2008) also addressed the potential of propiconazole to affect male fertility through anti-androgenic effects using Hershberger assay. The serum concentration of follicle stimulating hormone was significantly increased at 150 mg/kg bw/d. It was concluded that propiconazole had no antiandrogenic effects.

Moreover, the study by Tully *et al*. (2006) revealed no effects of 150 g/kg bw/d on testes weights or histology, sperm morphology or motility or any of the serum hormones measured (testosterone, LH, FSH, oestradiol). In contrast, when Wistar male rats were treated with 4 mg/kg bw/d propiconazole from post-natal day 50 to 120, a significant increase in abnormal sperm tail morphology, increased seminal vesicle and vas deferens weight, and decreased serum estradiol levels were observed (Costa *et al*., 2015).

Oestrous cyclicity was disrupted on first two weeks after vaginal opening in rats exposed to 500 ppm propiconazole from gestation day 6 through gestation, parturition, and lactation (Rockett *et al*., 2006). No effects on anogenital distance was reported in this study at doses comparable to those employed in Taxvig *et al*. (2008). The oestrous cyclicity was later normalized. It was concluded that exposure to high concentrations of propiconazole adversely impacted the reproductive development of the female rat. The effects appeared to be either short term or reversible.

Comparison with the criteria

Sexual function and fertility

The only available study assessing the effects of propiconazole on fertility and sexual performance was a 2-generation study showing no effects on mating, fertility, gestation, female and male fertility index and average of gestation length. However, other effects in F_1 and F_2 generations were reported as reductions in body weight, hepatotoxicity, reductions in testes weight and reductions in mean number of live pups (only in F_2).

Reductions in body weight and hepatotoxicity were consistently reported in most of the repeated dose toxicity studies. It suggests that the effects found in F_1 and F_2 of this 2-generation study might be due to systemic toxicity rather than a direct effect on reproduction. Thus, RAC does not consider hepatotoxicity in the F_1 and F_2 generation to be relevant for classification.

A reductions in the weight of the testes was reported for the second litter of both generations. No histopathological assessment of the altered testes was available, but it is notable that the chronic toxicity studies did not report this effect and there were no alterations of sexual and reproductive performance, which makes the biological significance of this finding questionable. Taking into consideration these facts, RAC does not consider the effects on testes relevant for classification.

Finally, reductions in the mean number of live pups of F_{2b} were reported in different period of lactation, these reductions were statistically significant and ranged between 19 and 26%. However, RAC notes that in F_{2a} also reductions in the mean number of live pups were reported but in different periods of lactation and that such reduction were not reported for any of the F_1 litters.

RAC notes that there are several studies in the open scientific literature reporting impairments in serum testosterone levels, testes and foetus weight, anogenital distance, oestrus cyclicity and sperm quality, suggesting endocrine mediated effects. However, RAC also notes that such observations did not alter fertility in the 2-generation Guideline study,that the reported effects are reversible in some cases and finally, effects reported in individual studies were not further confirmed in others with similar approaches. Thus, RAC does not consider the effects reported in these studies to be consistent enough to warrant classification.

In conclusion, RAC supports the DS proposal for **no classification of propiconazole for fertility effects.**

<u>Development</u>

The two available developmental studies in rat reported cases of cleft palate. Cleft palate occurred in 1/302 (0.33%) pups at 90 mg/kg bw/d without significant maternal toxicity, and 2 cases were seen at 360/300 mg/kg bw/d, although at this dose together with severe maternal toxicity (clinical signs). In a second independent study cleft palate was again observed at 300 mg kg bw/d (also together with maternal clinical sings: 17% reduction in corrected maternal body weight gain and 2 mortalities) with an incidence of 2/2061 foetuses (0.097%) from 2/158 litters. According to the CLH report, cleft palate had not been seen previously in the performing laboratory (incidence 0/5431 during 1983-1985) and according to data submitted by the registrant the observed incidences are also above the historical control data of other laboratories during 1983-1986 (4/25522, 0.016%). RAC notes that cleft palate is a serious malformation that should be taken into consideration for classification purposes.

In addition to the cleft palate, other developmental effects were reported in the rat studies. These were skeletal variations (rudimentary ribs and non-ossified sternebrae) at 90 mg/kg bw/d and 360/300 mg/kg bw/d, and increased incidence of urinary tract variations at 360/300 mg/kg bw/d. These visceral findings appeared only at doses exerting maternal

toxicity and might be attributable to a secondary consequence of it, while the skeletal findings appeared with both, maternal (360/300 mg/kg bw/d) and non-maternal (90 mg/kg bw/d) toxicity and following a dose-response pattern and therefore should be considered for classification.

Other reported developmental effects were resorptions, abortions, early deliveries and increased incidence of fully formed 13th ribs in rabbits exposed at 400 mg/kg bw/d. However RAC notes that these effects appeared at doses causing maternal body weight gain reductions of 89% and 56% in the periods between 10-14 and 14-20 days of gestation, respectively. RAC considers these effects as additional concerns for classification of developmental toxicity.

There is no information about the potential toxicity of propiconazole for humans and therefore Category 1A is not supported.

A substance can be classified as reproductive toxicant category 1B on the basis of animal studies providing clear evidence of an adverse effect on sexual function and fertility or on development 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 other toxic effects.

Cleft palate is a severe malformation that can be induced by chemicals if the critical dose and timing of exposure are aligned. It has also been suggested that cleft palates could occur as a consequence of maternal toxicity. RAC notes that cleft palate appeared with low incidence, but in two independent studies and in different litters. RAC also notes that cleft palate appeared in the presence of severe maternal toxicity in two studies, but also at 90 mg/kg bw/d in the absence of relevant maternal toxicity and following a dose-response pattern (0.33% at 90 mg/kg bw/d and 0.70% at 300 mg/kg bw/d). These two facts (the appearance in two independent studies and the dose-response) speak against the cleft palates being chance findings and support their association with exposure to propiconazole. Furthermore, the increased incidence of cleft palates in rat has also been observed in response to exposure to other triazoles (e.g. cyproconazole and epoxiconazole).

The mode of action of propiconazole in the observed developmental alterations is not known, but the teratogenicity of triazoles is suggested to be related to altered embryonic retinoid acid catabolism, since abnormalities are confined to structures controlled by retinoid acid. There is no information showing that the mechanism is not relevant for humans and whether human sensitivity is more similar to rabbits (where no cases were reported) or to rats.

RAC noted that the cleft palate appeared only in rats and not in rabbit. However, RAC also notes that some cases might be masked by the post-implantation loss and the reduced number of viable foetuses in the rabbit study.

In conclusion, RAC considers increases in cleft palate incidences found in both rat developmental studies as of human relevance. The following findings also contribute to consider propiconazole as presumable developmental toxicant for humans: 1) skeletal variations at 90 mg/kg bw/d in rat study; and, 2) resorptions, abortions and early deliveries in rabbits exposed to 400 mg/kg bw/d.

RAC consequently proposes propiconazole to be classified as **reproductive toxicant category 1B H360D (May damage the unborn child).**

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

Not evaluated in this dossier.

4.11.1.2 Immunotoxicity

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 50: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis No guideline followed Principles of method other than guideline The test substance was incubated for up to 28 days at a range of pH values at 70 °C; the solutions were then analysed for hydrolysis products using GC.	No significant hydrolysis	Specific activity: 59.6 µCi/mg	Burkhard N (1980a) DAR IIA 2.9.1/01 DAR IIA 7.2.1.1/01
Direct photolysis US EPA Guideline subd. N,161-2	DT50: 249 days (12 h irradiation/12 h dark per day)	Artificial sunlight (12h light/12 h dark), intensity (506 Watts/m^2) compared to natural sunlight. Unlabelled: Purity: 90% [phenyl-(U)-^{14}C] - propiconazole: Radiochemical purity: 97.03%; Specific activity: 40 uCi/mg	Das YT (1990) DAR IIA 2.9.2 DAR IIA 7.2.1.2
Ready biodegradation OECD Guideline 301 B	0-3% degradation of test substance Not readily biodegradable	Purity : 91.1%	Bader U (1990) DAR IIA 7.2.1.3.1
Biodegradation in water and sediment – simulation test 1 Various: Dir. 95/36/EC and guidelines for the approval of plant protection products, Part IV, 5-1, Degradability and fate of plant protection products in water / sediment system, Federal Biological Research Centre for Agriculture and	DT50 : 5.5 — 6.4 days in freshwater at 20°C 485 — 636 d in entire system at 20 °C	Unlabelled Purity: 98.80% [^{14}C] - CGA64250: Radiochemical purity: 97.4%; Specific activity: 1.76 MBq/mg	Reischmann FJ (1999) DAR IIA 7.2.1.3.2

Method	Results	Remarks	Reference
Forestry, Federal Republic of Germany			
Aerobic mineralisation in surface water – simulation test 2 OECD Guideline 309	DT50: 78.29 days DT90: 260.1 days	dosed at a low concentration (10µg/mL) in freshwater at 20°C. Unlabelled Purity: 98.4% [chlorophenyl-U-^{14C}] - CGA64250: Radiochemical purity: 99.2%;	Matthews ME & Schaeffer EC (2013) DAR IIA 7.2.1.3.2/02

5.1.1 Stability

Hydrolysis

One hydrolysis study is available for propiconazole.

Study 1 (Burkhard, 1980a)

The study was non-GLP with no specific guidelines followed. The chemical hydrolysis of ^{14}C-triazolring labelled propiconazole was investigated in different aqueous media with pH values 1, 5, 7, 9, and 13 and at a concentration of 10 ppm. The test solutions (not sterilized) were incubated in brown flasks and thermostatistically controlled-water-bath rotary shakers 28 days at 70 °C. Samples were analysed by gas chromatography. Hydrolysis products were not identified. No significant hydrolysis of the test substance occurred at each pH-value at 70 °C over a period of 28 days. The total recovery was determined to be 100 ± 3.1 %. The amount of propiconazole left at the end of the study was 94-106 % of the original radioactivity.

Photolysis in water

One direct photolysis study is available showing that propiconazole undergoes very limited photodegradation.

Study 1 (Das, 1990)

Following GLP and to EPA subd. N, 161-2 guidelines, the photolysis of phenyl ^{14}C radiolabelled propiconazole was assessed at 25±1°C in sterile buffer solution at pH 7. A xenon lamp, filtered for wavelengths 300-800 nm, was used to irradiate the samples using 12 hour light(irradiated)/12 hour dark(non-irradiated) cycles for a period considered by the applicant to be equivalent to 30 days natural summer sunlight at test location (545.8 W/m^2).

Under non-irradiated conditions parent concentrations ranged from 95.5 -97.9% nominal, with no significant change over the study period. Under irradiated conditions the parent concentrations decreased from an initial concentration of 97.9% to a final concentration of 88.4%. There were no major degradates under irradiated or non-irradiated conditions. Four minor radioactive components (unknowns 1-4) were discernible in the radiochromatogram of the irradiated solution, reaching a maximum value of 3.4% (unknown 1) by 21 days. Under non-irradiated conditions, a maximum value of 1.3% (unknown 4) was recorded by 30 days. None of the minor metabolites exceeded 4.9 % AR.

The photolytic half-life was 249 days. It can be concluded that propiconazole is relatively stable and photolysis in water is not a major degradation pathway.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Estimation not applicable as a study is available.

5.1.2.2 Screening tests

Study 1 (Bader, 1990)

A ready biodegradation test to GLP and following OECD Guideline 301B resulted in 3% degradation (based on theoretical carbon dioxide) within 28 days. Propiconazole concentrations were 10.9 mg/l and 20.7 mg/l in the test and the blank control of the emulsifier and the reference substance were included in the test. During the study 0 % of propiconazole biodegradation occurred at the lower concentration and 3 % in the higher. On this basis, it is concluded that propiconazole is not readily biodegradable.

5.1.2.3 Simulation tests

Study 1 (Reischmann, 1999)

A GLP, water/sediment study is available which followed Commission Directive 95/36/EC Annex II: 7.2.1.3.2 Water/Sediment Study and Various: Dir. 95/36/EC and guidelines for the approval of plant protection products, Part IV, 5-1, Degradability and fate of plant protection products in water / sediment system, Federal Biological Research Centre for Agriculture and Forestry, Federal Republic of Germany. Natural sediment/water samples (river and pond) were treated with ^{14}C-labelled test substance at a concentration corresponding to a field rate of 0.127 kg/ha and incubated at 20°C over a period of 175 days. The sampling dates were 0, 14, 33, 63, 90, 119, and 175 days after treatment in both pond and Rhine systems.

The total recovery ranged from 98.1 -107.4% of applied radioactivity for all samples. Propiconazole dissipated moderately quickly from the water phase of aquatic systems with half-lives between 5.5 (Rhine water) and 6.4 days (pond water). Propiconazole adsorbed in the sediment in large amounts and only dissipation half-life in water was reported but no degradation half-life in water. Therefore, the degradation half-life from the whole water/sediment system is used. For the total aquatic system, the DT50 values were 485 days (pond) and 636 days (river). Up to eight minor metabolites were detected with maximum concentrations, with none exceeding 5% of the applied radioactivity. Volatile products evolved were ≤ 0.4% of the applied radioactivity. The non-extractable radioactivity of the pond sediment increased to 7.9% (day 90) and was 7.6% at the end of incubation and for river sediment was 10.1% (day 90) and 9.1% (day 175). The results are presented in the following tables.

Table 51: Balance of radioactivity applied to pond and Rhine river water aquatic system treated with propiconazole (values are given % in the applied radioactivity

Incubation days	Volatiles	Water	Extrables	Non-extrables	Recovery

	Pond	Rhine	Pond	Rhine	Pond	Rhine	Pond	Rhine	Pond	Rhine
0	n.p.	n.p.	98.1	96.5	2.0	2.0	0.4	0.4	100.6	98.9
14	<0.1	0.2	22.5	16.3	80.2	81.6	3.1	2.6	105.8	100.7
33	0.2	0.3	10.7	10.0	91.0	88.7	3.2	4.4	105.1	103.3
63	0.2	0.3	8.4	6.7	88.7	87.9	4.4	4.8	101.7	99.7
90	0.3	0.4	9.9	7.6	89.3	86.2	7.9	10.1	107.4	104.2
119	0.3	0.3	8.3	8.6	90.3	87.7	7.3	9.3	106.2	105.9
175	0.4	0.4	7.1	8.4	83.0	87.5	7.6	9.1	98.1	105.3

Table 52: Distribution pattern of 14C-propiconazole in pond and Rhine river aquatic systems (values are given in % of the applied radioactivity)

Pond aquatic system			Rhine river aquatic system		
Incubat. days	Compartment	Propiconazole	Incubat. days	Compartment	Propiconazole
0	Water	98.1	0	Water	96.5
	Sediment	2.0		Sediment	2.0
14	Water	20.0	14	Water	15.1
	Sediment	79.7		Sediment	81.6
33	Water	7.4	33	Water	7.0
	Sediment	91.0		Sediment	85.5
63	Water	2.9	63	Water	3.2
	Sediment	84.4		Sediment	84.2
90	Water	2.7	90	Water	2.9
	Sediment	85.6		Sediment	81.9
119	Water	1.2	119	Water	2.3
	Sediment	87.0		Sediment	80.3
175	Water	0.9	175	Water	2.0
	Sediment	76.8		Sediment	81.7

Study 2 (Matthews & Schaeffer, 2013)

In a GLP study, aerobic mineralisation in surface water following OECD 309 was determined with application of [^{14}C-chlorophenyl]-labelled propiconazole in Tuckahoe Lake natural water. Radio-labelled test substance was applied to the water at nominal rates of 10 and 95 µg/L (low and high, respectively). The 95 µg/L rate was also applied to sterilized test systems (sterilized natural water). The systems were incubated in the laboratory under aerobic conditions. Samples were subjected to diffuse, fluorescent light (12 hr light/dark cycle) or maintained in continuous darkness at 20 ± 2°C for 62 days.

Significantly more degradation of the test substance was observed in natural water dosed at a low concentration (10 µg/L) and subjected to diffuse light than in low dose test vessels (10 µg/L) subjected to continuous darkness. 1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol (CGA91305) was the only major metabolite found, reaching a maximum level of 41.8% of applied radioactivity in low dose test vessels (10 µg/L) subjected diffuse light. Degradation of the test substance was seen only at very low levels or not at all in high dose test vessels (95 µg/L) in the presence or absence of light. Thus, a degradation rate for the high dose samples could not be determined due to an insufficient number of data points.

Mineralization in low dose samples treated at 10 µg/L appeared to have occurred during the study.

Approximately 1% AR per interval appeared to be produced as mineralization in the low dose vessels. With numerous sampling intervals, the 1% AR values compounded to make it appear as if high mineralization (11%) had occurred in the low dose vessels by day 62. This was speculated to be derived from natural background as evidenced by the fact that activity observed in the KOH trapping solution was nearly the same for all vessels regardless of dose level or degradation pattern. Additionally, chemiluminescence was said to be known to occur in alkaline solutions particularly in KOH which was used as the trapping solution in this study. Results for the low dose samples was thought to reflect exaggerated $14CO^2$ levels because the natural background is proportionately larger in comparison to the total radioactivity present than in high dose samples where the total radioactivity present was nearly ten times higher. It was argued that contrary to the values reported, mineralization likely occurred at much lower levels or not at all in the vessels dosed at 10 µg/L. Further, the exaggerated 14CO2 levels artificially contributed to the total % AR recovered causing mass balances to be higher than normal in some cases.
Mineralization was a very minor route of degradation with CO_2 accounting for < 2% AR in vessels dosed at 95 µg/L. Mineralization was therefore considered to be a minor route of degradation.

Estimation of the degradation rate (DT50) was only possible for the low dose samples subjected to diffuse light. The DT50 value in surface water was calculated (using non-linear regression and a single first-order kinetic model (SFO, CAKE, Version 1.4) to be 78.29 days for the low dose vessels (10 µg/mL). The DT50 value is summarized in the following table.

Table 53: DT50 value for propiconazole treated samples exposed to diffuse light

System	Test concentration (µg/L)	SFO				
		$DegT_{50}$ (days)	k	Chi^2	R^2	Prob > 1
Tuchkahoe Lake Natural Water	10	78.29	0.008854	3.345	0.7694	2.00E-05

5.1.3 Summary and discussion of degradation

The test substance was not significantly hydrolysed when incubated at 70°C for up to 28 days in the pHs 1,5,7, 9 and 13. Therefore propiconazole is considered hydrolytically stable under environmentally relevant conditions.

The photolytic half-life (aquatic) was 249 days and therefore photolysis is not considered to contribute significantly to the degradation of propiconazole in water.

Propiconazole is considered not readily biodegradable according to the available study.

In water and sediment test systems, propiconazole dissipated moderately from the water phase of aquatic systems with half-lives between 5.5 and 6.4 days. Propiconazole adsorbed in the sediment in large amounts and only dissipation half-life in water was reported but no degradation half-life in water. Therefore, the degradation half-life from the whole water/sediment system is used. For the total aquatic system, the DT50 values were 485 days (pond) and 636 days (river). Up to eight minor metabolites were detected with maximum concentrations not exceeding 5% of the applied radioactivity for any of them. Mineralisation was only a minor element of dissipation of propiconazole in aquatic water/sediment systems. On this basis propiconazole is not considered to

undergo rapid ultimate degradation. Consequently propiconazole is considered not rapidly degradable for the purposes of classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 54: Adsorption desorption studies

Method	Results					Remarks	Reference
Adsorption/ desorption in various soil types EPA (Subdivision N, Series 163-1, 1982)	Soil	Absorption		Desorption		^{14}C-Propiconazole Radiochemical purity: 97.9% (determined by sponsor); 97.7% (determined at HLA) Specific activity: 52.1 uCi/mg	Saxena AM (1988) DAR IIA 7.1.2/01
		Equilibrium constant (k_d)	Sorption coefficient (K_{oc})	Equilibrium constant (k_d)	Sorption coefficient (K_{oc})		
	Plainfield	1.20	685	1.57	893		
	Mississipi	2.81	436	3.00	464		
	California	4.49	382	5.35	455		
	Hagerstown	8.88	604	10.3	703		
	Arizona	9.34	1134	10.1	1229		
	Absorption equilibrium constants (k_d) for propiconazole : varied between 1.2 and 9.3. Desorption equilibrium constant and Sorption coefficient(K_{oc}) : varied between 1.6 and 10.3.						
No guidelines followed. (see remarks)	Soil	Absorption		Desorption		100 mg analytical grade CGA64250 containing approx.. 5% ^{14}C-labelled CGA64250 Specific activity: 59.6 µCi/mg	Burkhard N (1980b) DAR IIA 7.1.2/02
		k (µg/g soil)	Q (µg/g organic material)	k (µg/g soil)	Q (µg/g organic material)		
	Vetroz	59.03	1059	70.75	1263		
	Les Evouettes	26.20	728	31.65	879		
	Collombey	8.48	385	10.57	480		
	Lakeland	10.96	913	15.98	1329		
	Freundlich absorption constants (k) : varied between 8.48 and 59.0µg/g soil. The desorption constants: varied between 10.6 and 70.8µg/g soil. Soil was mixed with aqueous solutions of test substance; absorption on to soil was calculated from the difference between the initial and equilibrium concentrations of test substance in the aqueous phase. Additional water was added to the treated soil, after 3 days mixing the equilibrium concentration was determined; desorption from soil was calculated from the difference between the initial and equilibrium concentrations of test substance in the aqueous phase.						

Two studies are available on adsorption and desorption on representative agricultural soils.

Study 1 (Saxena, 1988)

The absorption of ^{14}C-labelled propiconazole was assessed in five different soils following EPA Guideline Subdivision N, Series 163-1 (1982) and in compliance with GLP. Results are presented in the table above.

Study 2 (Burkhard, 1980b)

The absorption of ^{14}C-propiconazole (CGA64250) was assessed in four soils following no specific guideline. The test was done before GLP. Although there were some deficiencies (e.g. too short shaking time, no duplicate measurements in different concentrations, mass balance not calculated) in the study it is considered acceptable because it gave corresponding results with the other study (Saxena 1988) which was conducted according to EPA guidelines and in GLP. Results are presented in the table above.

Summary

Table 55: Summary of KFOC and 1/n (Freundlich exponent) adsorption values for propiconazole in different soils

Soil	pH	Organic matter (%)	Organic carbon (%)	Adsorption			Reference
				K_F (mL/g)	K_{FOC} (mL/g)	1/n	
Collombey	7.8	2.2	1.28	8.48	665	0.86	Burkhard, 1980
Vetroz	6.7	5.6	3.25	59.03	1817	0.88	
Les Evouettes	6.1	3.6	2.09	26.20	1255	0.81	
Lakeland	6.3	1.2	0.70	10.96	1575	0.85	
Sand (USA)	5.4	0.3	0.17	1.20	690	0.895	Saxena, 1988
Silt loam (USA)	7.0	1.1	0.64	2.81	440	0.899	
Sandy loam (USA)	7.5	2.0	1.16	4.49	387	0.894	
Silty clay loam (USA)	6.8	2.5	1.45	8.88	612	0.831	
Clay loam (USA)	7.8	1.4	0.81	9.34	1150	0.841	
Arithmetic mean				**15.0**	**955**	**0.86**	

5.2.2 Volatilisation

Propiconazole is considered poorly volatile. The vapour pressure and Henry's law constant for propiconazole are 5.6×10^{-5} Pa at 25°C and 9.2×10^{-5} Pa m^3 mol^{-1}, respectively. Volatilisation of propiconazole from soil was not significant and from plant surfaces ~30% at 20°C over a 24 hour period.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

Table 56: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Bioconcentration: Flow-through Fish Test Bluegill sunfish, *Lepomis macrochirus.* OECD Guideline 305	Lipid content: 2.48 — 4.91 % (days 1, 21, 28, 42 days) (Total fish, controls) 3.03 — 3.31 % (days 1, 21, 28, 42 days) (Total fish, high dose) 3.66 — 4.18 % (days 1, 21, 28, 42 days) (Total fish, low dose) The mean measured bioconcentration factors for the test substance residues in non-edible portions, edible portions and whole fish were 322, 30 and 180, respectively The calculated kinetic mean BCFs for the test substance residues in non-edible portions, edible portions and whole fish : 332, 27 and 181, respectively	Unlabeled Purity: 98.2% [Triazole-(U)-^{14}C] CGA 64250 Radiochemical purity: 97% Specific activity: 0.238 MBq/mg	DAR IIA 7.2.1.3

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

5.3.1.2 Measured bioaccumulation data

A 28 days aquatic bioaccumulation study to GLP and following OECD Guideline 305 is available

Study 1 (DAR IIA 7.2.1.3)

The uptake, depuration and bioconcentration of ^{14}C- propiconazole in bluegill sunfish (*Lepomis macrochirus*) were investigated in a 28-day flow-through exposure and 14-day transfer to clean water. The test was run according to the OECD 305 guideline and GLP. A continuous flow delivery system was used to supply two test vessels with test item, prepared in ethanol, at a nominal concentrations of 0.064 and 0.0064 mg/L and a solvent control vessel with 0.001 mL/L ethanol.
Test substance residues were rapidly concentrated in fish tissues, reaching a stable steady-state concentration within about 17 days for the higher and the lower concentration. Measured bioconcentration factors (as a mean of the lower and the higher concentration) for the test substance residues in non-edible portions, edible portions and whole fish were 322, 30 and 180, respectively. The calculated BCFs from the kinetic data (as a mean of the lower and the higher concentration, again) for the same tissues were 332, 27 and 181, respectively. The depuration time for 90% of the bioconcentrated radioactivity for the whole fish was less than 1 day for the higher and less than 2

days for the lower concentration, respectively.
Lipid content of the test organisms was determined for edible portions, non-edible portions and for the whole fish. However, the results were expressed in relation to edible portions, non-edible portions and for the whole fish but not in relation to the determined lipid content. Mean water temperatures (of all vessels) were between 22.0 °C and 22.5 °C except a time of 6 hours on day 8 when it was 16.5 °C in all vessels. Although the stability of the temperature is one of the validity criteria of the OECD guideline 305 the fluctuation in the temperature was so short-term and therefore the study is considered valid.

5.3.2 Summary and discussion of aquatic bioaccumulation

The steady-state whole fish bioconcentration factor for *Lepomis macrochirus* was 180. On the basis of the bioaccumulation in fish study with BCFs less than 500 propiconazole is not considered bioaccumulative for classification purposes.

5.4 Aquatic toxicity

Key studies considered valid for classification are presented in the Table 57with additional information presented in the relevant sections below. A full set of valid acute and chronic fish, invertebrate and algae studies are available for propiconazole. Based on short term studies the most sensitive species is *Americamysis bahia* (EC_{50} 0.51 mg/l) and the lowest chronic value was obtained for the fish *Cyprinodon varieagatus* (NOEC 0.068 mg/l).

The major metabolite found in the biodegradation test for surface water (1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol, CGA91305) has been demonstrated to be less toxic than propiconazole (Table 58) and, therefore, has not been taken into account any further in this classification proposal.

Table 57: Summary of relevant information on aquatic toxicity

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Short term toxicity to fish						
Propiconazole (92.4%)	*Oncorhynchus mykiss*	OECD 203 92/69/EEC C.1 EPA OPP 72-1	96 hr LC50 96 hr NOEC	4.3 mg/L 1.0 mg/L	Static (based on nominal, measured stayed between 80-120%)	DAR IIA 8.2.1/02
Propiconazole (90.7%)	Leiostomus xanthurus	OECD 203	96 hr LC50 96 hr NOEC	2.6 mg/L 0.93 mg/L	Static (mean measured)	DAR IIA 8.2.1/01
Long term toxicity to fish						
Propiconazole (95.2%)	*Pimephales promelas*	OECD Draft proposal (2002) OECD 229 EPA OPPTS 850.1500 OPPTS 890.1350	235 day NOAEC	0.188 mg/L	Flow-through (mean measured)	dRAR IIA 8.2.2.1/01
Propiconazole (91.7%)	*Pimephales promelas*	EPA OPPTS 72-4	NOEC EC10 (wet length) EC20 (wet length) EC10 (length) EC20 (length)	0.43 mg/L 0.38 mg/l 0.47 mg/l 0.49 mg/l 0.68 mg/l	Flow-through (mean measured)	DAR IIA 8.2.2.1/04 dRAR 8.2.2.1/01
Propiconazole (91.7%)	*Cyprinodon variegatus*	US EPA OPPTS 850.1075	NOEC EC10 (number of eggs/d) EC20 (number of eggs/d)	**0.068 mg/L** 0.06 mg/l 0.10 mg/l **Key study**	Flow-through (mean measured)	DAR IIA 8.2.2.1/02 dRAR 8.2.2.2/01
Propiconazole (92.4%)	*Oncorhynchus mykiss*	OECD 204	21 d LC50 21 d NOEC (lethal effects) 21 d NOEC (non-lethal effects	1.1 mg/L 0.31 mg/L 0.31 mg/l Supportive study	Flow-through (mean measured)	DAR IIA 8.2.2.1/03
Propiconazole (95.2 %)	*Pimephales promelas*	OECD 229	NOEC (based on visual observation by the Dossier submitter)	0.12 mg/l	Flow-through (mean measured)	dRAR 8.2.2/02
Short term toxicity to aquatic invertebrates						

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Propiconazole (92.4%)	*Daphnia magna*	OECD 202 EPA OPP 72-2	48 hr EC50 48 hr NOEC	10.2 mg /L 4 mg/L	Static (based on nominal, measured stayed between 80-120 %)	Grade R, 1999a DAR IIA 8.2.4/01
Propiconazole (90.7%)	*Americamysis bahia)*	Test method not specified Validity evaluated under Directives 98/8/EC and 91/414/EEC and EU Regulation 528/2012	96 hr LC50	**0.51 mg/L** **Key study**	flow-through (mean measured)	Hollister T, 1981a DAR IIA 8.2.4/02
Long term toxicity to aquatic invertebrates						
Propiconazole (90.7%)	*Daphnia magna*	US EPA 1975	21 d NOEC	0.31 mg/l Supportive study	flow-through (mean measured)	LeBlanc G and Mastone J,1981 DAR IIA 8.2.5/01
Propiconazole (90.7 %)	*Mysidopsis bahia*	Not speicified in the report	28 d	NOEC 0.114 mg/l Supportive study	flow-through (mean measured)	Hollister, T.A. 1981b DAR IIA 8.2.5
Propiconazole (95.2%)	*Daphnia magna*	OECD 211 OCSPP Guideline 850.1000 OCSPP Draft 850.1300	21 d EC50 21d NOEC (reproduction) 21d NOEC (total body length)	1.0 – 1.1 mg/L 0.73 mg/L 0.37 mg/L	semi-static (mean measured)	Fournier A, 2014 dRAR IIA 8.2.5.1/01
Toxicity to algae and cyanobacteria						
Propiconazole (95.2%)	*Pseudokirchneriella subcapitata*	OECD 201 EPA OPPTS 850.5400 Commission Regulation (EC) No 761/2009 C.3 JMAFF Test Guidelines, 2-7-7	72 h EbC50 72 h ErC50 72 h EyC50 NOErC NOEbC, NOEyC	1.6 mg/L 9.0 mg/L 1.0 mg/L 0.46 mg/L 0.13 mg/L	Static (mean measured)	Hoger S, 2011 DAR IIA 8.2.6/01
Sediment Dwelling Toxicity Test						
Propiconazole (92.4%)	*Chironomus riparius*	OECD Guideline 218 & 219	28 d EC50 emergence rate 28 d EC50 development rate	9.5 mg/L 35.5 mg/L	Static (based on nominal, measured stayed between 80-120 %)	Grade R, 1999b DAR IIA 8.2.7/01

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
			28 d NOEC emergence rate 28 d NOEC development rate	8.0 mg/L 4.0 mg/L		

Table 58: Summary of relevant information of major metabolite CGA091305 on aquatic toxicity

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
(1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol, CGA91305 (99 ± 2 %)	*Oncorhynchus mykiss*	OECD 203	96 hr LC_{50}	24 mg/L	Static (based on nominal, measured stayed between 80-120 %)	dRAR 8.2.1/01
(1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol, CGA091305 (99 ± 2 %)	*Daphnia magna*	OECD 202	48 hr EC50	110 mg/L	Static (based on nominal, measured stayed between 80-120 %)	Wallace 2001b dRAR 8.2.4.1/02
(1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol, CGA091305 (99 ± 2 %)	(R116857) Algae *Selenastrum capricornutum*	EPA OPPTS 850.5400	72 hr E_bC_{50} 72 hr E_rC_{50}	9.6 mg/L 19.1 mg/L	Static (based on nominal, measured stayed between 80-120 %)	Wallace & Woodyer 2001 dRAR 8.2.6.1/05

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1 (DAR IIA 8.2.1/02)

The acute toxicity to the rainbow trout (*Oncorhynchus mykiss*) was determined under static conditions following guideline OECD 203 (equivalent to 92/69/EEC C.1 and EPA OPP 72-1). Dechlorinated tap water was used as blank control and the oxygen content, pH and temperature was measured daily in each test chamber. The nominal exposure concentrations of propiconazole (purity 92.4%) were 1.0, 1.8, 3.2, 5.8 and 10 mg test item/L. The measured concentrations ranged from 100-109% of the nominal ones at the start of the test and 89 – 104% at the end of the test. Based on the nominal concentrations, the 96h LC50 was 4.3 mg/L. Sub-lethal effects were observed at nominal concentrations above 1.0 mg/L and the 96 h NOEC was 1.0 mg/L.

Study 2 (dRAR 8.2.1/01)

The acute toxicity to spot marine (*Leiostomus xanthurus*) was determined under static conditions following OECD Guideline 203. Dissolved oxygen and pH were measured 0, 24, 48 and 96 hours after dosing and temperature and salinity were measured daily in one seawater control container. Dissolved oxygen was 74 % of saturation in all aerated treatments. One fish died in the vehicle control but in the blank control there was no mortality during the test, The nominal concentrations of propiconazole (purity 90.7%) were 0.65, 1.1, 1.8, 3.0 and 5.0 mg test item/L, and the corresponding mean measured concentrations were 0.53, 0.93, 1.52, 2.84 and 5.02 mg test item/L. Based on mean measured concentrations, the 96h LC50 was 2.6 mg/L. Sub-lethal effects were observed at mean measured concentrations above 0.93 mg /L and the 96 h NOEC was 0.93 mg/L.

5.4.1.2 Long-term toxicity to fish

Five long-term toxicity studies are available.

Study 1 (dRAR 8.2.2.1/01)

A life-cycle toxicity study to fathead minnow (*Pimephales promelas)* was determined under flow-through conditions following guidelines OECD Draft proposal (2002), OECD 229, EPA OPPTS 850.1500 and OPPTS 890.1350. The nominal concentrations of propiconazole (purity 95.2%) were 9.0, 26, 78, 233 and 700 µg/L, the corresponding mean measured concentrations were 7.9, 21, 63, 188 and 558 µg/L. The biological endpoints evaluated in the first generation (F0) were survival, fecundity, fertility and spawning frequency. The second generation (F1) endpoints measured were survival, hatching success, photographic length at Day 28 and 56 post-hatch; and lengths, weights and histological sex on all thinned fish at Day 192 post-hatch (at formation of reproductive groups). Reproductive fish from the F1 generation were monitored for the following endpoints: egg production, spawning frequency, lengths, weights, histological determination of sex, secondary sex characteristics, plasma vitellogenin concentration, and gonadal and liver histopathology. Survival and hatching success were monitored for the F2 generation.

Observations and measurements of fathead minnow were used to estimate the overall mean measured test concentration producing no adverse effect on the exposed organisms (No-Observed-Adverse Effect Concentration, NOAEC).Based on mean measured concentrations and effects on reproductive endpoints in the F0 and F1 generation, the NOAEC for propiconazole was 0.188 mg/l.

Study 2 (DAR IIA 8.2.2.1/04; dRAR 8.2.2.1/01)

The toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae was determined under flow-through conditions following guidelines EPA OPPTS 72-4. Well water was used in the test and properties of water were measured weekly, pH and oxygen content and temperature daily. The concentrations of propiconazole and acetone were determined on the test days 0, 4, 11, 18, 21, 25, 32 and 53. The nominal concentrations of propiconazole (purity 91.7%) were 0.063, 0.13, 0.25, 0.50, 1.0 mg/L, the corresponding mean measured concentrations were 0.069, 0.12, 0.21, 0.43, 0.97 mg/L. Based on mean measured concentrations, the 35 day MATC was >0.43 - < 0.97 mg/L. Sub-lethal effects were observed at mean measured concentrations at and above 0.97 mg/L and 35 day NOEC was 0.43 mg/L.

The original study (DAR IIA 8.2.2.1/04) did not provide estimates for the EC10 and EC20 for the response variable of hatchability, post-hatch survival, larval wet weight at test termination and larval length at test termination. Therefore in accordance to the recommended test guidelines set out in Commission Communication 2013/C95/01 the data was re-analysed (dRAR 8.2.2.1/01) in order to provide these values. As a result of statistical analyses of hatchability and post-hatch survival revealed no reliable significant difference between the control and treatment groups, therefore the EC_{10} and

EC_{20} values could not be calculated. However, statistical analyses of the weight and length measurements revealed significant differences when compared to the control and EC_{10} and EC_{20} values were calculated resulting EC_{10} (95% CL) of 0.38 (0.32 - 0.43) mg/L and EC_{20} (95% CL) of 0.47 (0.41 – 0.52)mg/L for wet length and EC_{10} (95% CL) of 0.49 (0.49 – 0.49) mg/L and EC_{20} (95% CL) of 0.68 (0.68 – 0.68) mg/L for length.

Study 3 (DAR IIA 8.2.2.1/02; dRAR 8.2.2.2/01)

The chronic toxicity to sheepshead minnow (*Cyprinodon variegatus)* was determined under flow-through conditions following guidelines US EPA, OPPTS 850.1075. The effects of propiconazole on hatching success, survival, growth and reproductive success of first generation (F0) of sheepshead minnow and the hatching success, survival and growth of their progeny (F1) was studied in for 100 days (95 days F0 exposure, 91 days post-hatch F0 exposure, five additional days to complete the F1 exposure). All the water quality parameters were determined before the test. Temperature and dissolved oxygen were measured daily and salinity and pH weekly. The nominal concentrations of propiconazole (purity 91.7%) were 0.019, 0.038, 0.068, 0.15, 0.30 and 0.60 mg/L with the corresponding mean measured concentrations 0.016, 0.038, 0.068, 0.15, 0.29 and 0.55 mg/L. Based on mean measured concentrations the NOEC (reproduction) was 0.068 mg/L.

The original study (DAR IIA 8.2.2.1/02) did not provide estimates of the EC_{10} and EC_{20} for the response variables embryo hatching success (F_0, F_1 generations), survival, reproductive success and length and weight of both F_0 and F_1 generations. Therefore in accordance to the recommended test guidelines set out in Commission Communication 2013/C95/01 the data was re-analysed (dRAR 8.2.2.2/01) in order to provide these values.

Statistical analysis of the F_0 female fry weights at 91 days post-hatch revealed an EC_{10} value of 0.44 mg/L and an EC_{20} value of 0.47 mg/L. It was not possible to determine confidence intervals for these values due to the absence of a meaningful dose response, therefore the reliability of the estimates is questionable.

Statistical analysis of the number of eggs/female/day produced by F_0 females revealed an EC_{10} of 0.06 mg/L (95% c.l. 0.00-0.12 mg/L) and an EC_{20} of 0.10 mg/L (95% c.l. 0.01-0.15 mg/L).

Statistical analysis of all remaining parameters revealed no significant differences between control and treatment groups, therefore no EC_{10} or EC_{20} values were calculated.

Study 4 (DARIIA 8.2.2.1/03)

The prolonged toxicity to rainbow trout (*Oncorhynchus mykiss)* was determined under flow-through conditions following guideline OECD 204. The nominal concentrations of propiconazole (purity 92.4%) were 0.031, 0.093, 0.28, 0.83, 2.5 mg/L, the corresponding mean measured concentrations were 0.038, 0.081, 0.31, 0.95, 2.8 mg/L. Mean measured concentrations ranged from 87 % to 123 % of the nominal concentrations and the results were based on the actual mean concentrations after 0, 7, 14 and 21 days. According to the test report deviations from the OECD guideline 204 (1984) were: average size of the fish was 61 mm (53-68 mm) based on 70 fish on day 0 and oxygen content was below 60 % (57, 58, 59 %) on day 6 (at 0.95 mg/l),16 (at 0.31 mg/l) and 20 (at 2.8 mg/l), respectively. Based on mean measured concentrations of propiconazole the 21-day LC_{50} was 1.1 mg/L. The mortality was 20 % and 100 % in the two highest concentrations 0.95 mg/l and 2.8 mg/l, respectively. It is noted that oxygen content was 57 % on the day 6 when one fish died in the concentration of 0.95 mg/l. Growth was the most sensitive parameter as non-lethal effects and showed effects at the concentration of 0.95 mg/l, but was not affected in the next lower concentration of 0.31 mg/l. In the original study report NOEC for lethal effects was claimed to be 0.95 mg/l although 20 % of the fish died in that concentration. This was said to be based on statistical analysis but no details on the analysis were given. Therefore, the 21-day NOEC for both lethal and non-lethal effects is considered to be 0.31 mg/l. However, due to identified deficiencies the study is considered only as a supportive study.

Study 5 (dRAR 8.2.2.2/02)

The toxicity of propiconazole (purity 95.2 %) to reproductively active group of fathead minnow (*Pimephales promelas*, approximately 22 weeks old) was determined during 21 days under flow-through conditions following OPPTS Guideline 890.1350 and OECD Guideline 229. Fish were exposed to the nominal concentrations of 0.012, 0.12 and 1.2 mg/l and a dilution water control, the corresponding mean measured concentrations were 0.010, 0.12 and 1.0 mg/l. . Four replicate vessels were established for each treatment level. Test vessels were maintained at the test conditions specified in the protocol: a temperature of 25 ± 1 ºC, photoperiod of 16 hours of light at an intensity range of 540 to 1100 lux (50 to 100 footcandles) and 8 hours of darkness. Samples were removed from two replicates (A and C) of each treatment level and the control at day 0 (exposure initiation) and day 10 and from two replicates (B and D) on days 3 and 18.

No abnormal behaviour or notable changes in secondary sex characteristics were observed throughout the 21-day study. Statistically significant decreases were determined for fecundity, fertilization success, female vitellogenin and female survival; statistically significant increases in male and female gonadal somatic indices (GSI) were also noted. A statistically significant increase in fecundity was observed at the 0.12 mg a.s./L treatment although this was not considered to be of biological significance. A significantly increased incidence of oocyte atresia was observed in the gonadal histopathological findings.

There were statistically significant effects on reproduction, fertility, GSI, female vitellogenin concentration and female survival in addition to observed increased incidences of oocyte atresia in fish exposed to treatment levels of 1.0 mg/L. No NOEC value was given in the study report. However, based on visual observation of the result table presented in the original study report the Dossier submitter is considering NOEC value to be 0.12 mg/l for fegundity (number of eggs/female).

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Two studies assessing the short-term toxicity of propiconazole to aquatic invertebrates are available.

Study 1 (Grade, 1999a)

The acute toxicity of propiconazole (purity 92.4%) to water flea (*Daphnia magna*) was assessed following GLP and to OECD Guideline 202 and EPA OPP 72-2 in a static test design for 48 hours. The oxygen content, pH and temperature were measured at the start and at the end of the test. The nominal exposure solutions were 0.5, 1.0, 2.0, 4.0, 8.0 and 16 mg/. The measured concentrations were 95-103 % of the nominal ones at the start of the test and 93-105 % at the end of the test. There was no immobilisation of daphnids observed in the controls (blank and vehicle) throughout the test. Based on nominal concentrations, the 48 hour EC50 was 10.2 mg/L. Sub-lethal effects were observed at nominal concentrations at and above 8 mg/L and 48 hour NOEC was 4 mg/L.

Study 2 (Hollister, 1981a)

The acute toxicity of propiconazole (purity 90.7%) to marine mysids (*Americamysis bahia*) was assessed in an intermittent flow-through test design for 96 hours. Test method was not specified, but the method applied was claimed to be in conformity with international regulatory requirements for assessing the acute toxicity of chemicals to shrimps. The validity of the test has been evaluated under Directives 98/8/EC and 91/414/EEC and under EU Regulation 528/2012. The test was done before GLP requirements but it was otherwise satisfactorily performed and reported although the report did not include data concerning light conditions and no blank control was used. Test organisms were obtained from Cultured at Bionomics Marine Research Laboratory. Shrimps were acclimated before the test in natural sea water and 20 shrimps (age 6-8 days) were used per dose level (five test concentrations and vehicle control). The water parameters were measured daily and oxygen concentration was > 84 % of the saturation at the end of the study. Samples for chemical analysis of propiconazole were taken from alternate dublicates of each treatment at the beginning and at the end of the study. The nominal concentrations of propiconazole were 188, 375, 750, 1,500 and 3,000 µg/L, the average measured concentrations were 158, 456, 753, 1,402, 2,900 µg/L (the measured values ranged 70 to 89 % of the nominal ones at the end of the test). Mortality and general symptoms of toxicity were recorded 24, 48, 72 and 96 hours after dosing. Based on mean measured concentrations, the 96 hour LC50 was 0.51 mg/l (conf. limit 0.4 - 0.7).

5.4.2.2 Long-term toxicity to aquatic invertebrates

Two studies assessing the long-term toxicity of propiconazole to aquatic invertebrates are available.

Study 1 (LeBlanc & Mastone, 1981)

The chronic toxicity of propiconazole (purity 90.7%) to water flea (*Daphnia magna*) was assessed following US EPA 1975 Guideline in a flow-through test design for 21 days. The temperature and dissolved oxygen were measured every weekday and total hardness, alkalinity, specific conductance and pH of the test solution within one replicate aquarium from each treatment and from the controls. The concentration of propiconazole at different treatment levels and controls was checked prior to the test and weekly during the exposure. Adult survival was determined weekly and determinations of offspring production were made on weekdays from day 7 through 21. The nominal concentrations of propiconazole ranged 50-86 % of the measured ones and were 0.10, 0.20, 0.40, 0.80, 1.6 mg/L, the corresponding mean measured concentrations were 0.05, 0.14, 0.31, 0.69, and 1.3 mg/L. Based on

mean measured concentrations, the 21 day NOEC was 0.31 mg/L. According to the PPPD monograph the test did not exactly correspond to the guidelines e.g. mortalities were not recorded daily and the results were not reported detailed enough (e.g. EC50). In addition, the chemical analysis of propiconazole was not satisfactorily reported. Therefore the study is used only as a supportive study for the classification purpose.

Study 2 (Fournier, 2014)

The effect of propiconazole (purity 95.2 %) on the survival, growth, reproductive output and offspring sex ratios of the water flea, *Daphnia magna* was studied during 21 days. The exposure was conducted under static renewal conditions following OECD Guideline for Testing of Chemicals 211, OCSPP Draft Guideline 850.1300 and OCSPP Guideline 850.1000. Ten replicate vessels, each containing one daphnid (aged < 24 h), were established for each treatment level and the control. Nominal concentrations for the treatment levels tested were 0.20, 0.41, 0.80, 1.6, and 3.2 mg/L (0.18, 0.37, 0.73, 1.5 and 2.9 mg/L as mean measured concentrations). Exposure solution concentrations were analytically confirmed in newly prepared test solutions on days 0, 2, 9, 16 and 19 and in aged exposure solutions on days 2, 5, 12, 19 and 21. Test vessels were maintained in a temperature-controlled environmental chamber set at 20 ± 1 °C with a 16 hour light and 8 hour dark photoperiod at a light intensity of 2.3 to 12 µE·m-2·s-1 (170 to 890 lux). The number of immobilized adult daphnids and observations of abnormal behavior in each test vessel were recorded daily. Numbers of offspring were determined upon the first brood release in any vessel and daily throughout the remainder the test. In addition, the number of immobilized offspring and the time to first brood release were recorded for each treatment level and the control. The female to male ratio in the second brood of offspring for each concentration and control was also assessed. At exposure termination (day 21), the total body length of each surviving adult daphnid was measured and dry weights were determined. The results are based on mean measured concentrations and 21 d NOEC values for survival, reproduction, total body length and total dry weight were 1.5, 0.73, 0.37 and 0.73 mg/l, respectively. No adverse effect was observed for sex ratio.

Study 3 (Hollister, 1981b)

The chronic toxicity of propiconazole (purity 90,7 %) to mysid shrimp (*Mysidopsis bahia*) was investigated in a flow-through system for 28 days. The study was not conducted according to GLP and no specific guidelines were followed. The vehicle control was included in the study and 20 shrimps (< 48 h old) were used in each concentration level. Salinity, temperature, pH and dissolved oxygen were measured daily and water samples for chemical analyses of propiconazole were taken alternatively from duplicates of each treatment level on days 0, 7, 14, 21 and 28. Nominal concentrations were 0.062, 0.125, 0.25, 0.5 and 1.0 mg a.i./l. The measured concentrations of propiconazole after different time intervals ranged from 49 to 122% of the nominal values and the mean measured concentrations were 82-101% of the nominal concentrations. The dissolved oxygen was 60-90 % of saturation throughout the study, pH was 7.8-7.9 and the temperature 26-28 °C. NOEC for reproduction was determined to be 0.114 mg/l. The light conditions were not reported. The results were quite briefly reported for instance reproduction results only after 28 days and the mortality was given only as percentages but not given individually mortality data on each time interval. The measured concentrations were under 80% of the nominal concentrations. There was only vehicle control but no seawater control and the mortality in the other duplicate of the vehicle control was 20%. Therefore, the study is used only as a supportive study for classification purpose.

5.4.3 Algae and aquatic plants

Three studies assessing the toxicity of propiconazole to various algae were available. Two of them, studies on *Navicula seminulun* and *Skeletenoma costatum* were considered unacceptable already

during the assessment of propiconazole under EU biosides and Plant protection products legislations, and therefore, are not used for this CLH proposal.

Study 1 (Hoger, 2011)

A 96 hour static algal growth inhibition study of propiconazole (purity 95.2%) following GLP and to guidelines OECD 201 (equivalent to EPA OPPTS 850.5400, Commission Regulation (EC) No 761/2009 C.3 and JMAFF 2-7-7) to unicellular green algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is available. The preparation of the test media was based on the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, 2000. The nominal dilutions of propiconazole were 1:1600, 1:500, 1:160, 1:50, 1:16, 1:5, the mean measured concentrations were 0.042, 0.13, 0.46, 1.37, 4.63 and 14.7 mg/L (81-95 % of initial measured concentrations). All validity criteria mentioned in the OECD guideline 201 were met.

The algal cell densities were measured at 24, 48, 72 and 96 hours and the biomass integral (area under the growth curve, AUC), growth rate and yield were calculated. The results are based on the mean measured concentrations calculated as the geometric means of the concentrations measured at the start and end of the test. The test item had a significant inhibitory effect on the biomass integral and yield after the test period of 72 hours at the measured concentration of 0.46 mg/L and at all higher test concentrations. The growth rate was statistically significantly reduced after 72 hours at the mean measured concentration of 1.37 mg/L and above. The 72-hour NOEC was determined to be 0.13 mg/L for biomass integral and yield, and 72-hour NOEC for growth rate was 0.46 mg/l. The 72-hour EC50 values for the inhibition of biomass integral (AUC), average growth rate and yield were 1.6 mg/L, 9.0 mg/L and 1.0 mg/L respectively.

The ErC50 value of 9.0 mg/l and the 72-hour NOErC of 0.46 mg/l are used for the classification purpose.

5.4.4 Other aquatic organisms (including sediment)

One study assessing the sediment toxicity of propiconazole is available.

Study 1 (Grade, 1999b)

The toxicity of propiconazole (purity 92.4%) to *Chironomus riparius* was assessed in a full life cycle toxicity test to GLP and following OECD 218 & 219 guidelines in a static test for 28 days in two exposure scenarios (A and B).

Scenario A (spiked water): The test substance was added to a water column of sediment-water systems containing 20 first instar larvae under static conditions. Approximately 28 hours after addition of the test organisms, the test substance was introduced by pipette below the surface into the water column of the test system at nominal concentrations of 0.25. 0.50. 1.0. 2.0, 4.0, 8.0 and 16.0 mg/L.

Scenario B (spiked sediment): The test substance was mixed with treated sand with aged artificial sediment at nominal concentrations of 25, 50, 100, 200 and 400 mg test item/kg sediment (dry weight) prior to introduction of *Chironomus* larvae. Spiked sediment and water were added to the test vessels about 48 hours prior to test initiation.

The biological assessment was based on impacts on full maturation of the larvae to adult midge. Main parameters examined were the rate and time of emergence and the total number of fully emerged male and female midges.

Based on the nominal concentrations, the values for A (spiked water) as follows:

- Emergence rate: EC50 9.5 mg/L, NOEC 8.0 mg/L, LOEC 16 mg/L
- Development rate: EC50 35.5 mg/L, NOEC 4.0 mg/L, LOEC 8.0 mg/L

Based on the nominal concentrations, the values for B (spiked sediment) as follows:

- Emergence rate:123 mg/kg (95% CL 91- 245), NOEC 25 mg/kg, LOEC 50 mg/kg
- Development rate: >100 mg/kg, NOEC 50 mg/kg, LOEC100 mg/kg

The concentrations of the test substance in the sediment were from 84 to 95% of the nominal concentrations. NOEC = 25 mg/kg dry sediment (nominal concentration). In order to convert it to the TGD default wet weight of suspended sediment it has to be divided by a factor of 4.6. The concentration in freshly deposited sediment is considered to be more relevant to aerobic sediment dwelling organisms than the concentration in lower sediment layers, and therefore, the factor for suspended sediment is used in the conversion. Consequently, NOEC = 25/4.6 = 5.4 mg a.i./kg wet sediment.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Propiconazole is considered hydrolytically stable under environmentally relevant conditions for the purposes of classification.

A ready biodegradation test resulted in 3% degradation (based on theoretical carbon dioxide) at day 28. On this basis, it is concluded that propiconazole is not readily biodegradable. Mineralisation was only a minor element of dissipation of propiconazole aquatic water/sediment systems. On this basis propiconazole is not considered to undergo rapid ultimate degradation and is considered not rapidly degradable for the purposes of classification.

The log Pow of 3.72 is lower than the trigger value of 4 for Regulation EC 1272/2008.

In the water/sediment study up to eight minor metabolites were detected with maximum concentrations not exceeding 5% of the applied radioactivity for any of them, In the biodegradation test for surface water one major metabolite (1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol) was found, reaching a maximum level of 41.8% of applied radioactivity in low dose test vessels (10 µg/L). However, that metabolite has been demonstrated to be less toxic than propiconazole and therefore this classification proposal is based only on propiconazole ecotoxicity

A full set of valid acute fish, invertebrate and algae/aquatic plant data are available for propiconazole. Based on the available acute toxicity data for fish, aquatic invertebrates and algae, the lowest acute toxicity value, is an EC_{50} of 0.51 mg/l for *Americamysis bahia* which is between 0.1 and ≤1 mg/L leading to an M factor of 1. Based on chronic aquatic toxicity data of fish, aquatic invertebrates and chronic NOEC for algae, the lowest chronic toxicity value, is a NOEC of 0.068 mg/l for *Cyprinodon varieagatus* which is between 0.01 and ≤ 0.1 mg/L leading to an M factor of 1 for this not rapidly degradable substance. Consequently the classification as Aquatic Acute 1, M-factor 1 and Aquatic Chronic 1, M-factor 1 is applicable.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

CLP:	**Aquatic Acute 1; H400, M-factor = 1** **Aquatic Chronic 1; H410, M-factor = 1**

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

Propiconazole has currently the following classification as hazardous to the aquatic environment in Annex VI to CLP: Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

The current DS's proposal for consideration by RAC was Aquatic Acute 1 (H 400) with an M-factor of 1 and Aquatic Chronic 1 (H410) with a separate M-factor of 1. The proposal was based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to aquatic invertebrates and fish regarding acute and chronic aquatic toxicity, respectively. Based on the available acute aquatic toxicity data for fish, aquatic invertebrates and algae, the lowest acute aquatic toxicity value is an EC_{50} of 0.51 mg/L for *Americamysis bahia,* which is between 0.1 and ≤1 mg/L leading to an M-factor of 1. Based on chronic aquatic toxicity data for fish, aquatic invertebrates and algae, the lowest chronic aquatic toxicity value is a NOEC of 0.068 mg/L for *Cyprinodon variegatus,* which is between 0.01 and ≤ 0.1 mg/L leading to an M-factor of 1 for this non-rapidly degradable substance. Consequently, the DS concluded that classification as Aquatic Acute 1, M-factor 1 and Aquatic Chronic 1, M-factor 1 is warranted.

The impurities were taken into consideration by the DS in the classification of this substance but none of them were found to be relevant for the classification.

Degradation

Propiconazole was not significantly hydrolysed when incubated at 70°C for up to 28 d at pHs 1, 5, 7, 9 and 13, and thus, it is considered hydrolytically stable under environmentally relevant conditions. Photolytic half-life was 249 d in a study following GLP principles and EPA subd. N, 161-2 guideline, and therefore, photolysis in water is not considered to be a major degradation pathway.

A ready biodegradation test (OECD TG 301B) resulted in 3% degradation (based on theoretical carbon dioxide) at day 28. On this basis, it is concluded that propiconazole is not readily biodegradable.

In biodegradation simulation studies, the DT_{50} values of propiconazole in aquatic water/sediment systems were 485-636 d for the whole system (Dir. 95/36/EC Annex II: 7.2.1.3.2 and guidelines for the approval of plant protection products, Part IV, 5-1BBA, Germany) and in surface water 78 d or higher (OECD TG 309). In the water/sediment study up to eight minor metabolites were detected with maximum concentrations not exceeding 5% of the applied radioactivity for any of them. In the surface water study, one major metabolite (1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1- yl)ethanol) was found, reaching a maximum level of 41.8% of applied radioactivity in low dose test vessels (10 µg/L).

Mineralisation was only a minor element of dissipation and degradation in the simulation studies. On this basis propiconazole is not considered to undergo rapid ultimate degradation.

Consequently, the DS concluded that propiconazole is considered not rapidly degradable for the purposes of classification.

Bioaccumulation

Propiconazole has a measured log Kow of 3.51-3.8 (EEC A.8, shake flask and HPLC methods), which is lower than the trigger value of 4 for substances with bioaccumulation potential according to the criteria in the CLP Regulation (EC 1272/2008).

A 28 d aquatic bioaccumulation study according to GLP principles and following OECD TG 305 is available. The steady-state whole fish bioconcentration factor for *Lepomis macrochirus* was 180 L/kg.

The DS concluded that on the basis of the bioaccumulation in fish study with BCFs less than 500 L/kg, propiconazole is considered not bioaccumulative for classification purposes.

Aquatic Toxicity

Acute and chronic aquatic toxicity data are available for the three trophic levels (fish, aquatic invertebrates and algae). The aquatic invertebrate *Americamysis bahia* was the most sensitive organism for acute aquatic toxicity and the fish *Cyprinodon variegatus* was the most sensitive organism for chronic aquatic toxicity.

As mentioned above in the degradation section, a major metabolite was observed in the biodegradation test with surface water. The metabolite was demonstrated to be less toxic than propiconazole, and therefore, it was not taken into account further in the classification proposal.

Table. Relevant aquatic toxicity data on propiconazole. The key study values triggering the classification are given in bold.

Method, test substance	Test organism	Conditions	Endpoint	Toxicity value (mg/L)	Reference
Acute toxicity to fish					
OECD TG 203 92/69/EEC C.1 EPA OPP 72-1	*Oncorhynchus mykiss*	Static nom	96 h LC_{50} 96 h NOEC	4.3 1.0	DAR IIA 8.2.1/02
OECD TG 203	*Leiostomus xanthurus*	Static mm	96 h LC_{50} 96 h NOEC	2.6 0.93	DAR IIA 8.2.1/01
Chronic toxicity to fish					
OECD Draft proposal (2002) OECD TG 229 EPA OPPTS 850.1500	*Pimephales promelas*	Flow-through mm	235 d NOAEC (reproduction)	0.188	dRAR IIA 8.2.2.1/01

OPPTS 890.1350					
EPA OPPTS 72-4	*Pimephales promelas*	Flow-through mm	NOEC EC_{10} (wet length) EC_{20} (wet length) EC_{10} (length) EC_{20} (length)	0.43 0.38 0.47 0.49 0.68	DAR IIA 8.2.2.1/04 dRAR 8.2.2.1/01
US EPA OPPTS 850.1500	*Cyprinodon variegatus*	Flow-through mm	NOEC (reproduction) EC_{10} (no. of eggs/d) EC_{20} (no. of eggs/d)	**0.068** 0.06 0.10 **Key study**	DAR IIA 8.2.2.1/02 dRAR 8.2.2.2/01
OECD TG 204	*Oncorhynchus mykiss*	Flow-through mm	21 d LC_{50} 21 d NOEC (lethal effects) 21 d NOEC (non-lethal effects	1.1 0.31 0.31 Supportive study	DAR IIA 8.2.2.1/03
OECD TG 229 OPPTS Guideline 890.1350	*Pimephales promelas*	Flow-through mm	21 d NOEC (no. of eggs/female) (based on visual observation by the Dossier submitter)	0.12	dRAR 8.2.2/02
Equivalent to OECD TG 229	*Pimephales promelas*	Flow-through nom	21 d NOEC (no. of eggs/female)	0.05	Skolness *et al.*, 2013
Acute toxicity to aquatic invertebrates					
OECD TG 202 EPA OPP 72-2 Under GLP conditions	*Daphnia magna*	Static nom	48 h EC_{50}	10.2	Grade, 1999a DAR IIA 8.2.4/01
Test method not specified Validity evaluated under Directives 98/8/EC and 91/414/EEC and	*Americamysis bahia*	flow-through mm	96 h LC_{50}	**0.51** **Key study**	Hollister, 1981a DAR IIA 8.2.4/02

EU Regulation 528/2012					
Modified ASTM, 2006 E-2455-06	*Lampsilis siliquoidea*	-	96 h EC_{50}	10.01	Bringolf, *et al.*, 2007
chronic toxicity to aquatic invertebrates					
US EPA 1975	*Daphnia magna*	flow-through mm	21 d NOEC	0.31 Supportive study	LeBlanc and Mastone,1981 DAR IIA 8.2.5/01
Method not speicified in the report	*Mysidopsis bahia*	flow-through mm	28 d NOEC	0.114 Supportive study	Hollister, 1981b DAR IIA 8.2.5
OECD TG 211 OCSPP Guideline 850.1000 OCSPP Draft 850.1300	*Daphnia magna*	semi-static mm	21 d NOEC (reproduction) 21 d NOEC (total body length)	0.73 0.37	Fournier, 2014 dRAR IIA 8.2.5.1/01
No standard guideline provided	*Daphnia magna*	-	21 d NOEC (development)	0.5	Kast-Hutcheson *et al.*, 2001
Toxicity to algae and cyanobacteria					
OECD TG 201 EPA OPPTS 850.5400 Commission Regulation (EC) No 761/2009 C.3 JMAFF Test Guidelines, 2- 7-7	*Pseudokirchneriella subcapitata*	Static mm	72 h EbC_{50} 72 h ErC_{50} 72 h EyC_{50} NOErC NOEbC, NOEyC	1.6 9.0 1.0 0.46 0.13	Hoger, 2011 DAR IIA 8.2.6/01
ASTM, 1996 Vol 11.05	*Dunaliella tertiolecta*	-	96 h ErC_{50} 96 h NOErC	2.33 0.375	Baird and De Lorenzo, 2010
nom = nominal concentration (measured maintained within 80-120 %) mm = mean measured concentration					

Table. Relevant aquatic toxicity data on the major metabolite CGA091305.

Method, test substance	Test organism	Conditions	Endpoint	Toxicity value (mg/L)	Reference
OECD TG 203	*Oncorhynchus mykiss*	Static nom	96 h LC_{50}	24	dRAR 8.2.1/01
OECD TG 202	*Daphnia magna*	Static nom	48 h EC_{50}	110	Wallace, 2001b dRAR 8.2.4.1/02
EPA OPPTS 850.5400	*Selenastrum capricornutum*	Static nom	72 h EbC_{50} 72 h ErC_{50}	9.6 19.1	Wallace & Woodyer, 2001 dRAR 8.2.6.1/05
nom = nominal concentration (measured maintained within 80-120 %)					

Fish

Two acute aquatic toxicity tests on fish were included in the CLH dossier, both carried out according to OECD TG 203. The lowest acute aquatic toxicity value for fish was an LC_{50} (96 h) of 2.6 mg/L for *Leiostomus xanthurus*.

Four chronic aquatic toxicity studies and one 21 d prolonged acute study on fish according to different standard guidelines are available. The lowest key study value, according to US EPA OPPTS 850.1500, resulted in a NOEC (reproduction) of 0.068 mg/L for *Cyprinodon variegatus*. In this study the effects of propiconazole on hatching success, survival, growth and reproductive success of first generation (F0) of sheepshead minnow and the hatching success, survival and growth of their progeny (F1) was studied for 100 d (95 d F0 exposure, 91 d post-hatch F0 exposure, five additional days to complete the F1 exposure).

Aquatic invertebrates

Two acute aquatic toxicity tests on aquatic invertebrates were provided. The lowest acute toxicity value is an LC_{50} (96 h) of 0.51 mg/L for the marine mysid *Americamysis bahia.* The test method was not specified but the method applied was claimed to be in conformity with international regulatory requirements for assessing the acute aquatic toxicity of chemicals to shrimps. The validity of the test has been evaluated under Directives 98/8/EC and 91/414/EEC and under EU Regulation 528/2012. The test was done prior to GLP requirements and the report did not include data concerning light conditions and no blank control was used. Nevertheless, as an extended explanation of the test conditions was included in the report, this test was considered by the DS to be reliable and the resulting LC_{50} as the lowest relevant acute value triggering the classification.

Regarding the chronic information on aquatic invertebrates, three chronic aquatic toxicity tests were included in the CLH dossier. The lowest reliable value was a NOEC (21 d) of 0.37 mg/L for body length for freshwater *Daphnia magna* obtained in a test carried out according

to the OECD TG 211. 21 d NOEC values obtained in this study for survival, reproduction and total dry weight were 1.5, 0.73 and 0.73 mg/l, respectively. All results are based on mean measured concentrations. No adverse effect was observed for sex ratio. The other two chronic studies on *Daphnia magna* and *Mysodopsis bahia* resulted in slightly lower values (21 d NOEC of 0.31 mg/L and 28 d NOEC of 0.114 mg/l, respectively) but they were only used as supportive information for the classification due to the lower reliability of these studies.

Algae and aquatic plants

One reliable algal study with *Pseudokirchneriella subcapitata* carried out according to OECD TG 201 was included in the CLH dossier. The study resulted in an E_rC_{50} (72 h) of 9.0 mg/L and a NOE_rC (72 h) of 0.46 mg/L.

Other aquatic organisms (including sediments)

Information on other aquatic organisms was also included in the CLH dossier. A study on the sediment-dwelling phase of the midge *Chironomus riparius* was provided. The toxicity of propiconazole (purity 92.4%) was assessed in a full life cycle toxicity test according to GLP and following OECD TG 218 & 219 in a static test for 28 d in two exposure scenarios: spiked water and spiked sediment.
Based on the nominal concentrations, the values for spiked water are as follows:

- Emergence rate: EC_{50} 9.5 mg/L; NOEC 8.0 mg/L.
- Development rate: EC_{50} 35.5; NOEC 4.0 mg/L

Based on the nominal concentrations, the values for spiked sediment are as follows:

- Emergence rate: EC_{50} 123 mg/Kg; NOEC 25 mg/kg.
- Development rate: EC_{50} > 100 mg/kg; NOEC 50 mg/kg

The study is considered not relevant for the classification of propiconazole as it is not a pelagic test. However, the data indicate a low level of toxicity and was included as additional confirmatory information for the aquatic compartment.

Comments received during public consultation

Two MSCAs commented during the public consultation supporting the DS's proposal for the environmental classification and M-factors. In addition, one MSCA expressed general support for the DS's proposal for classification.

During public consultation, additional ecotoxicological information on synergistic effects and potential endocrine disruption effects in fish was provided. RAC evaluated this additional information. However, RAC notes that this new information does not change the classification proposed by the DS.

Assessment and comparison with the classification criteria

Degradation

Propiconazole is hydrolytically and photolytically stable, is not readily biodegradable (3% degradation) and shows slow ultimate degradation in water/sediment and surface water simulation tests (DT_{50} values of 485-636 d and 78 d, respectively). Therefore, RAC agrees

with the DS's proposal that propiconazole is considered not rapidly degradable for the purposes of classification and labelling.

Bioaccumulation

Propiconazole has a measured log Kow of 3.51-3.8, which is lower than the trigger value of 4 for substances with bioaccumulation potential according to the criteria in the CLP Regulation (EC 1272/2008). Based on the information provided in the CLH dossier, the substance showed some surface active properties, (47.5 - 59.0 mN/m based on OECD TG 115), which could result in uncertainties in additional estimations such as Log Kow. However, any effect is expected to be low (criterion for surface active substances < 60 mN/m). Furthermore, an experimental BCF and chronic aquatic toxicity data are available.
The steady-state whole fish bioconcentration factor for *Lepomis macrochirus* was 180 L/kg (from a 28 d aquatic bioaccumulation study according to GLP principles and following OECD TG 305). Results were not expressed in relation to lipid normalisation. According to the provided information, lipid content of experimental organisms ranged from 2.48 to 4.91%. Considering statistical differences of lipid content (20%) between treatment groups, a BCF of 284 L/kg based on the mean of the group with the lowest lipid content (3.17%) was re-calculated by RAC.

RAC agrees with the DS's proposal that propiconazole has a low potential for bioaccumulation based on a measured log Kow of 3.51-3.8 and an experimental lipid normalised BCF in fish of 284 L/kg.

Aquatic toxicity

The major metabolite (1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanol) identified in the degradation simulation test for surface water shows less toxicity than propiconazole, and therefore, the classification proposal is based only on propiconazole.

Reliable acute and chronic aquatic toxicity data on propiconazole is available for all three trophic levels.
Based on the available acute aquatic toxicity data for fish, aquatic invertebrates and algae, the lowest relevant acute aquatic toxicity value is a LC_{50} (96 h) of 0.51 mg/L for *Americamysis bahia.* This is below the classification threshold of 1 mg/L and in the range of $0.1 < L(E)C_{50} \leq 1$ mg/L leading to an acute M-factor of 1.

Based on the available chronic aquatic toxicity data for fish, aquatic invertebrates and algae, the lowest relevant chronic aquatic toxicity value is a NOEC of 0.068 mg/L for *Cyprinodon variegatus*. This is below 0.1 mg/L, which is the classification threshold for category Chronic 1 for non-rapidly degradable substances, and in the range of $0.01 < NOEC \leq 0.1$ mg/L) leading to a chronic M-factor of 1.

Additional long-term information on fish was provided during the public consultation. Skolness *et al.* (2013) showed potential endocrine activity of propiconazole on *Pimephales promelas* after 21 d exposure conditions similar to OECD TG 229. The cumulative number of eggs per female was significantly reduced at propiconazole concentrations of 1.0, 0.50, 0.05 and 0.005 mg/L. However, at the concentration of 0.05 mg/L no significant effect was observed. Hence, it was not possible to conclude that the effect observed at 0.005 mg/L was caused by exposure to propiconazole. Consequently, the 21 d NOEC was considered to be 0.05 mg/L. Neither fertility nor hatching success of the deposited eggs were affected by propiconazole. Endocrine disruption *per se* is of no relevance for classification according to

the current EU system, whereas the observed effects on reproduction (number of eggs) are relevant. However, as the test followed the method of a screening assay, it is only used as supportive information by RAC, and other available long-term tests were considered to be of higher relevance. Therefore, the value considered by the DS (NOEC of 0.068 mg/L for *Cyprinodon variegatus*) was considered as the lowest relevant chronic value for classification.

RAC notes that there is acute information for the chronically most sensitive trophic level, *i.e.* fish, but not for the chronically most sensitive species (*Cyprinidon variegatus*), which has chronic values one order of magnitude lower than the other fish. Acute data on this species could potentially influence the acute M-factor. However, no effects on embryo hatching or post hatch survival were observed at 0.55 mg/L in the chronic Fish Full Life Cycle (FFLC) study. As this value is higher than the LC_{50} of 0.51 mg/L from the mysid shrimp study, which is used for the acute classification, it does not seem probable that further testing would provide relevant additional information.

Two additional studies on aquatic invertebrates were provided during the public consultation regarding synergistic effects. However, in the opinion of the RAC this new information does not affect the conclusions on the classification as proposed by the DS.

During the public consultation, a study following the guideline ASTM 1996 Vol. 11.05 on the alga *Dunaliella tertiolecta* was provided. The study resulted in a 96h E_rC_{50} of 2.33 mg/L and a 96 h NOE_rC of 0.375 mg/L. However, the reliability of the results could not be fully assessed based on the provided information, e.g. it was not mentioned whether the results were based on measured or nominal concentrations. Therefore, the study is only used as supportive information.

Furthermore, RAC noted that the biocides dossier PT 8 of propiconazole included a study on *Scenedesmus subspicatus* with an EC_{50} of 0.058 mg ai/L and a NOEC of 0.016 mg ai/L. However, the test was carried out with a propiconazole formulation, and the composition of that formulation has since changed. The *Pseudokirchneriella subcapitata* algae study with the active ingredient (propiconazole), which is included as the key algae study in the classification proposal, was performed at a later stage and was used for the biocides dossiers PT7 and PT9 of the substance. Consequently, RAC agrees with the DS that the earlier *Scenedesmus subspicatus* study is not relevant for the current classification proposal.

RAC noted that the dRAR of propiconazole included a *Xenopus laevis* study (OECD TG 231), which resulted in a 21 d NOEC of 0.056 mg/L for metamorphosis. RAC took the *Xenopus laevis* study into account as a supportive study since valid data for other species at the same trophic level shall also be considered, also according to the CLP guidance. RAC noted that it supports the DS's proposal for the environmental long-term (chronic) hazard classification and M-factor.

Conclusion on classification

Based on the above information, RAC agrees with the DS's proposal that propiconazole fulfils the classification criteria for **Aquatic Acute 1 (H400)** with an **M-factor of 1** and **Aquatic Chronic 1 (H410)** with an **M-factor of 1**.

6 OTHER INFORMATION

This substance has been reviewed under EU biocides legislation (Directive 98/8/EC and Regulation (EU) 528/2012) and under EU Plant protection products legislations (Directive 91/414/EEC). In addition there is ongoing evaluation under EU Regulation 1107/2009 and 844/2012 performed by the Finnish Competent Authority.

7 REFERENCES

Note: Some of the citations in the text include Annex point (e.g. "DAR IIA, 5.1.1/01") only and not author names. These sources are included in the reference list with "Confidential" in the column "Author(s)".

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8 ANNEX: IPCS/ILSI FRAMEWORK FOR THE EVALUATION OF THE HUMAN HEALTH RELEVANCE OF A HYPOTHESISED MODE OF ACTION

The full framework for the evaluation of the human relevance assessment of the mouse liver tumours is given in Green *et al*, 2014. Included here is a summary of the principle elements of the framework.

Following dietary administration for up to 2 years, high doses of propiconazole resulted in an increased incidence of liver tumors in male CD-1 mice. The tumor incidence was statistically increased but within the range of historic controls at 850 ppm (i.e. an equivocal response in a 79-week study) and clearly higher than control values at 2500 ppm (a dose that exceeded the Maximum Tolerated Dose [MTD] in a 2-year study). This annex assesses a postulated mode of action (MOA) for these propiconazole-induced liver tumors using the framework developed by the IPCS and ILSI/HESI. Finally, the human relevance of the identified MOA is assessed.

8.1 Is the Weight of Evidence Sufficient to Establish the Mode of Action (MOA) in Animals?

After the key events within a postulated MOA have been described, the Hill (1965) criteria and the framework outlined by IPCS (Boobis *et al*., 2006) require that they be evaluated by a standardized weight of evidence evaluation. For the key events to be causally related to the formation of tumors, they must:

- Be supported by data showing strength, consistency and specificity of association of key events and tumor response
- Show dose-concordance of key events and dose levels that produce tumors
- Occur in a logical temporal sequence
- Be reproducible
- Demonstrate that alternative MOAs have been considered and are not operative
- Be plausible and consistent with the current state of knowledge of the relevant biological processes

A weight of evidence analysis for the animal MOA with propiconazole is described in the following sections.

8.1.1 Dose-concordance of key events

Table 8-1 summarises the dose-concordance of the associated and causal key events. Overall, there is good dose concordance of the proposed key events with tumor outcome. With increasing doses, an increasing number of the key events are observed. Critically, it is only at dose levels close to (500 ppm) or equivalent to the tumorigenic doses (850 ppm and 2500 ppm) that three of the causal key events are observed:

- Increased expression of pro-proliferative and anti-apoptotic gene *Gadd45β* (500 and 2500 ppm). This parameter was not affected at 100 ppm, and it was not evaluated at 850 ppm.
- Transient increase in hepatocellular proliferation (both 850 and 2500 ppm), with no effect at 500 ppm.
- Increased incidence of altered hepatic foci (both 850 and 2500 ppm), with no effect at 500 ppm.

The associative key events of increases in liver hepatocellular hypertrophy and liver weight were observed in a dose-responsive manner consistent with the proposed MOA. No effects on these parameters were observed at the low dose of 20 ppm from subchronic studies. At higher dose levels, these associative key events were affected consistently across multiple studies, and they occurred at dose levels at or below the tumorigenic dose levels of 850 and 2500 ppm.

Table 8-1: Summary of dose-concordance of associative and causal key events

Dose of propiconazole (ppm)	**CAR activation (Causal)**	**Induction of *cyp* gene expression/ increased CYP protein/activity (Associative)**	**Increased hepatocellular hypertrophy (Associative)**	**Increased liver weight (Associative)**	**Induction of pro-proliferative and anti-apoptotic genes (Causal)**	**Transiently increased hepato-cellular proliferation (Causal)**	**Altered hepatic foci (Causal)**	**Increased Incidence of Liver Tumors (Outcome)**
***In vitro* studies**								
N/A (*in vitro*)	Yes	Yes	N/A	N/A	N/A	Yes	N/A	N/A
***In vivo* studies**								
20	No data	No data	No	No	No data	No data	No	Not expected[c]
100	Yes[a]	Yes	Yes	No	No	No	No	No
500	Yes[a]	Yes	Yes	Yes	Yes	No	No	No
850	Yes[a]	Yes	Yes	Yes	No data	Yes	Yes	Yes[b]
2500	Yes[a]	Yes	Yes	Yes	Yes	Yes	Yes	Yes

a Inferred from observed increases in *cyp* gene expression and/or increased CYP protein/activity.
b Equivocal increase, within the range of historic control data.
c Presumed negative for liver tumors, as no effect at 100 or 500 ppm.

8.1.2 Temporal-concordance of key events

When the tumorigenic dose levels are considered, the observed effects on parameters associated with the key events occur in a logical, time-dependent manner consistent with the proposed MOA. The temporal concordance is summarized in Table 8-2.

The observed steps occur in a logical sequence, with the key events preceding the time of occurrence of liver hepatocellular adenomas and carcinomas. In particular:

- Activation of CAR, induction of CYP activities, increased hepatocellular hypertrophy, and increased liver weight occurred early (1-7 days), and remained consistently affected over time.
- The causal key event of hepatocellular proliferation was transiently affected, with significant increases at 1-7 days, but no measurable sustained increase above control at 14 days and longer.
- Induction of pro-proliferative and anti-apoptotic genes (e.g. *Gadd45β*) also occurred early (detected at 4 days) and remained consistently increased at later measurement times (30 and 90 days).
- A long-term consequence of these early key events included the postulated formation of altered hepatic foci (eosinophilic foci and/or eosinophilic foci of cellular change), which were only observed beyond one year.

An increase in liver tumors required 1.5-2 years before it was observed.

TABLE 8-2 Temporal-concordance of associative and causal key events at the tumorigenic dose levels of 850 – 2500 ppm propiconazole

Time	CAR activation (Causal)	Induction of *cyp* gene expression/ increased CYP protein/activity (Associative)	Increased hepatocellular hypertrophy (Associative)	Increased liver weight (Associative)	Induction of pro-proliferative and anti-apoptotic genes (Causal)	Hepato-cellular proliferation (Causal)	Altered hepatic foci (Causal)	Increased Incidence of Liver tumors (Outcome)
1-7 days	Yes[a]	Yes	Yes	Yes	Yes	Yes	No	No
14 days	Yes[a]	Yes	Yes	Yes	Yes[b]	No	No	No
28 days	Yes[a]	Yes	Yes	Yes	Yes	No	No	No
60 days	Yes[a]	Yes	Yes	Yes	Yes[b]	No	No	No
90 days	Yes[a]	Yes	Yes	Yes	Yes	No[c]	No	No
1 year	No data	No data	Yes	Yes	No data	No data	No	No
1.5-2 years	No data	No data	Yes	Yes	No data	No data	Yes	Yes

a Inferred from observed increases in *cyp* gene expression and/or increased CYP protein/activity.
b Inferred from observed increases on days 4, 30 and 90.
c Inferred from observed lack of increase in cell proliferation (PCNA) at 850 ppm at 9 weeks (Gerspach, 1999).

8.1.3 Reproducibility and consistency

Where parameters were measured in multiple studies, there is a high degree of reproducibility between studies and consistency between key events. The first key causal event in the postulated MOA, activation of mouse CAR, was demonstrated to occur *in vitro* in a direct CAR activation assay, and it was evident in multiple *in vivo* studies based on increases in *cyp2b* gene expression, Cyp2b protein levels, and/or Cyp2b enzymatic activity (PROD). The liver weight increases and associated hepatocellular hypertrophy were consistently observed in all of the *in vivo* studies. The transient increase in hepatocellular proliferation was observed in two independent experiments in two independent laboratories, with similar dose responses. Finally, eosinophilic altered hepatic foci were observed in two long-term mouse studies at dose levels that produced an equivocal response (850 ppm) or a clear increase (2500 ppm) in hepatocellular adenomas.

8.1.4 Biological plausibility

The liver is the most common target tissue affected in carcinogenicity studies in rodents (Gold *et al*., 2001). This may be due to the fact that the liver is the major site of metabolic processing of xenobiotics, as well as being the first organ exposed following absorption from the gastrointestinal tract (if administered orally, as in the case of the carcinogenicity studies with propiconazole). The induction of liver tumors in male mice subsequent to the activation of CAR is a comprehensively studied and characterised MOA for a number of compounds, including the archetypal CAR activator phenobarbital (Whysner *et al*., 1996; Meek *et al*., 2003; Holsapple *et al*., 2006), the potent mouse CAR activator TCPOBOP (Huang *et al*., 2005) and the insecticide sulfoxaflor (LeBaron *et al*., 2013). In addition, the stated MOA and the clear dependence on CAR activation as a causal key event is consistent with the data for cyproconazole, another triazole fungicide that caused liver tumors in mice but not in rats in a CAR-dependent manner (Peffer *et al*., 2007).

8.1.5 Alternative mode of action hypotheses

In addition to CAR activation, a number of alternative MOAs for induction of liver tumors in rodents and/or humans have been demonstrated (Cohen, 2010). These alternative MOAs, and the reasons why they can be excluded for propiconazole, are described below. In addition to these alternative MOAs, several publications have described detailed biochemical changes associated with propiconazole treatment in mice. These represent some theoretical investigations into the Mechanisms of Action (as opposed to the higher-level Mode of Action framework that is the focus of this document). For completeness, these are also summarized below and evaluated for how they fit with the MOA that is outlined for propiconazole.

DNA reactivity and mutagenicity: Propiconazole was negative in wide array of *in vitro* and *in vivo* genotoxicity assays (summarized in Shane *et al*., 2011a and WHO, 2006). A reported increase in mutant frequency with propiconazole treatment in BigBlue® Mice (Ross *et al*., 2009; Ross and Leavitt, 2010) has been re-evaluated in an independent assessment (Shane *et al*., 2011a, b). This evaluation led to the conclusion that the data do not support an increase in propiconazole -induced mutant frequency. The weight of evidence from all available studies indicates that propiconazole is not directly or indirectly reactive with DNA or mutagenic.

Peroxisome proliferation: Propiconazole produced little or no increase in lauric acid 12-hydroxylase activity and Cyp4a levels of protein in liver fractions of treated mice (Beilstein, 1998). Both of these markers are greatly increased by peroxisome proliferators and the weight of evidence indicates that propiconazole is not a peroxisome proliferator.

Aromatic Hydrocarbon Receptor (AHR) P450 induction: Propiconazole did not produce a large increase in EROD activity nor an increase in Cyp1a protein levels in liver microsomes of treated mice (Beilstein, 1998). Both of these markers are greatly increased by aromatic hydrocarbon receptor (AHR) activators and the weight of evidence indicates that propiconazole is not an AHR activator.

Estrogenic stimulation: In two ER binding studies, propiconazole did not bind to the estrogen receptors (ER) at most concentrations tested, and appeared to severely disrupt the assay at very high concentrations (10-3 M). These data were inconsistent with competitive binding; therefore, propiconazole is not a binder to the estrogen receptor (Willoughby, 2012a, b). Propiconazole was negative for estrogenic effects in an uterotrophic *in vivo* assay in the ovariectomized rat (Sawhney Coder, 2012). In combination with the lack of effects on estrogen-sensitive tissues in the wider toxicology database, the weight of evidence indicates that propiconazole does not show estrogenic potential.

Cytotoxicity and regenerative hyperplasia: Cell proliferation caused by propiconazole was transient and can be contrasted with the sustained regenerative cell proliferation and development of long-term fibrosis seen with classical hepatotoxic agents that induce regenerative hyperplasia such as chloroform and carbon tetrachloride (Louis *et al*., 1998; Larson *et al*., 1994a, b; Pereira, 1994). As an example, an increase in liver cell proliferation was observed for up to 159 days of treatment in a study with chloroform in B6C3F1 mice (Pereira, 1994), but no cell proliferation was observed beyond 7 days in the current studies with propiconazole. Therefore, the evidence does not support a finding of regenerative hyperplasia, which is the causal key event required for carcinogenesis to be produced as a secondary consequence of hepatotoxicity. In the *in vivo* mouse studies, a limited amount of hepatic necrosis (single cell or focal/multi-focal) plus chronic inflammatory cell infiltration was observed, and this descriptive finding was observed in a 60-day study in CD-1 mice (Weber, 1999). However, the severity of these findings was limited, and no increase in hepatocellular necrosis was observed after 104 weeks of treatment with propiconazole at 2500 ppm. Some *in vitro* or *in vivo* studies have demonstrated an increase in markers of hepatic oxidative stress following treatment with propiconazole or other CAR activators (Bruno *et al*., 2009; Nesnow *et al.*, 2011; Weber, 1999), likely as a consequence of microsomal P450 induction. Oxidative stress and subsequent micropathology changes have been considered to have a possible role in the overall MOA spectrum of effects associated with CAR activation (Holsapple *et al*., 2006; Lake, 2009). In contrast, the pattern of effects seen with propiconazole differs in outcome from classic cytotoxic carcinogens in that it did not cause a diffuse necrosis in the liver that progressed to regenerative hyperplasia and longer-term fibrotic changes in the liver, unlike cytotoxic liver carcinogens such as chloroform and carbon tetrachloride (Louis *et al*., 1998; Larson *et al*., 1994a; Larson *et al*., 1994b; Pereira, 1994). Therefore, the weight of evidence shows that a MOA involving cytotoxicity and a subsequent sustained regenerative cell proliferation is not operative with propiconazole .

Statins/altered cholesterol biosynthesis: Propiconazole was not designed to inhibit HMG-CoA reductase so this MOA is unlikely to be operating. Nevertheless, plasma cholesterol levels were decreased in mice by propiconazole treatment. The sites of action in the cholesterol synthesis and metabolism pathway that are theorized to cause this effect are thought to be different from the statins. Experiments with another triazole fungicide, cyproconazole, have shown that the effect of lower plasma cholesterol at a tumorigenic dose of 200 ppm was completely blocked in mice lacking the CAR receptor (Peffer *et al*., 2007). CAR receptor activation has been shown to play a role in regulation of lipogenesis, β-oxidation of fatty acids, gluconeogenesis and cholesterol/bile acid metabolism (Wada *et al*., 2009; Moreau *et al*., 2007; Stanley *et al*., 2006). Therefore it is likely that an alteration in cholesterol metabolism is also a consequence of CAR activation by propiconazole.

8.1.6 Uncertainties, inconsistencies and data gaps

The available data strongly support the proposed MOA for induction of mouse liver tumors by propiconazole (Figure 1), while excluding/being unsupportive of the alternative MOAs described in section 4.1.5. No uncertainties or inconsistencies have been identified. The only minor data gap is the lack of data concerning altered expression of pro-proliferative/anti-apoptotic genes for the 850 ppm dose level. This data gap does not affect the overall evaluation as it can be safely assumed that the changes observed at 2500 ppm and to a lesser extent 500 ppm also occur at 850 ppm, albeit to a lesser extent, as evidenced by the smaller magnitude of effects on liver enzyme induction, liver weight, hepatocellular proliferation and, ultimately, an equivocal increase in liver tumor incidence.

8.2 Assessment of the Postulated Mode of Action

The concordance analyses presented in sections 8.1.1 and 8.1.2 have established that the proposed key events resulting in the induction of liver tumors in male mice are reproducible across a number of studies and exhibit strong dose- and temporal-concordance with the tumor endpoint. This is a well described MOA for the induction of liver tumors in mice, and the parameters essential for describing the MOA have been demonstrated experimentally for propiconazole. Therefore, there is a high level of confidence that the hypothesised MOA (Figure 1) was responsible for the induction of liver tumors in male mice following dietary exposure to 850 ppm (equivocal increase) and 2500 ppm propiconazole.

8.3 Are the Key Events in the Animal Mode of Action Plausible in Humans?

Following establishment of a plausible MOA for the induction of liver tumors in mice, the next step is to assess the relevance to humans by assessing the qualitative and quantitative differences between the mouse and human for each of the key events. As described in a recent extension of the IPCS Mode of action Framework (Boobis *et al*., 2006), the questions to be asked are:

- can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?, and
- can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

8.3.1 Qualitative differences in key events

As described above, propiconazole was shown to be a potent direct activator of mouse CAR and a weak activator of human CAR *in vitro*. Therefore, it can be concluded that the human is not qualitatively different to the mouse with respect to the causal key event of CAR activation following propiconazole treatment, although a clear quantitative difference was observed.

To explore the species differences in response to propiconazole, an *in vitro* investigative study using primary hepatocytes isolated from male CD-1 mice was conducted to assess the effects of propiconazole on Cyp gene expression and hepatocellular proliferation (Elcombe, 2012), and a similar experiment was conducted with isolated male human hepatocytes (Elcombe, 2011). Table 8-3 provides a summary of the data from the two studies and shows that while treatment with propiconazole caused induction of Cyp gene expression (measured as an increase in *Cyp2b* and *Cyp3a* transcript levels) in both species, it only elicited a proliferative response in the mouse hepatocytes. The difference in proliferative response between the two species is indicative that in the mouse, but not the human, expression of pro-proliferative/anti-apoptotic gene(s) was increased, resulting in an increased cell proliferation only in mouse hepatocytes. The ability of the human hepatocytes to proliferate in response to appropriate stimuli was demonstrated by the use of epidermal growth factor (EGF) (Elcombe, 2011).

TABLE 8-3 Qualitative comparison of the effects of propiconazole and EGF on selected parameters in male CD-1 mouse and human hepatocytes *in vitro*

	Propiconazole		**EGF**	
	Mouse	**Human**	**Mouse**	**Human**
***Cyp2b* expression**	↑	↑	**N/A**	**N/A**
***Cyp3a* expression**	↑	↑	**N/A**	**N/A**
Hepatocellular proliferation	↑	---	↑	↑

Key: ↑ = Parameter significantly increased by treatment; --- = Parameter unaffected by treatment. Cell proliferation was measured via BrdU incorporation into hepatocytes.

Therefore, for propiconazole, it can be concluded that the human is similar to the mouse with respect to the associative key event of increased *Cyp2b* and *Cyp3a* gene expression, but is qualitatively different from the mouse with respect to the causal key event of increased hepatocellular proliferation following propiconazole treatment. A comparison of the key events in male mice and humans is presented in Table 8-4, which provides a summary of the species differences that have been demonstrated for the key events in this MOA.

TABLE 8-4 Species comparison of key events in mice and humans following exposure to propiconazole

Species	**CAR activation (Causal)**	**Induction of *cyp* gene expression/ increased CYP protein/activity (Associative)**	**Increased hepatocellular hypertrophy (Associative)**	**Increased liver weight (Associative)**	**Induction of pro-proliferative and anti-apoptotic genes (Causal)**	**Hepato-cellular proliferation (Causal)**	**Altered hepatic foci (Causal)**	**Increased incidence of liver tumors (Outcome)**
Male Mice	Yes (strong; 40- fold)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Humans	Yes (weak; 3-fold)	Yes[a]	Plausible	Plausible	Not likely[b]	No[a]	Not likely[b]	Not likely[b]

a Demonstrated in hepatocytes.
b Not likely based on lack of hepatocellular proliferation and known species differences with other CAR activators.

Human hepatocytes have been shown to be non-responsive to propiconazole regarding the causal key event of cell proliferation. This pattern of effects matches the known species differences that have been demonstrated for other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MOA for propiconazole between mice and humans. Therefore, it can be concluded that the tumorigenic MOA established for propiconazole in male mice is not operative in humans based on qualitative differences between mice and humans in their response to propiconazole.

This conclusion is supported by data for other CAR activating compounds; a review of these data across multiple species is available in Elcombe *et al*. (2013). Although CAR activators can increase liver size in both rodents and humans (Aiges *et al.,* 1980), significant species differences in the mitogenic and anti-apoptotic properties of such compounds have been demonstrated. In contrast to effects in cultured rodent hepatocytes, these compounds do not induce replicative DNA synthesis and do not inhibit apoptosis in human hepatocytes (Hasmall and Roberts, 1999; Hirose *et al*., 2009; Parzefall *et al*., 1991). Furthermore, a number of epidemiological studies have demonstrated that in human subjects receiving a CAR activating drug (phenobarbital) for many years at doses producing plasma concentrations similar to those that are carcinogenic in rodents, there is no evidence of increased liver tumor risk (IARC, 2001; Olsen *et al.,* 1989, 1995; Whysner *et al.,* 1996; Friedman *et al.,* 2009).

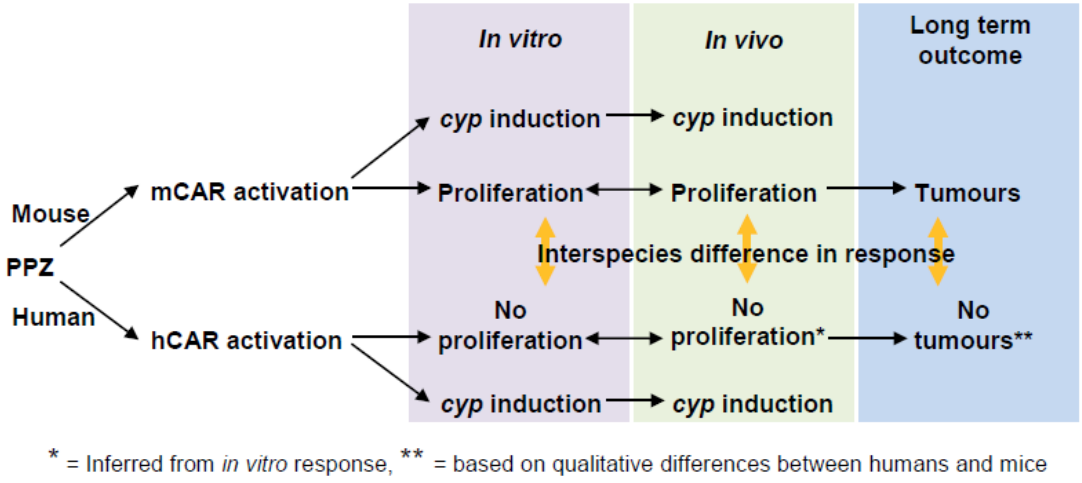
Therefore, the answer to the question "Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?", is clearly "Yes" for propiconazole.

Considering that a qualitative difference has been established, the question of quantitative differences in key events between experimental animals and humans does not need to be reviewed in this assessment.

8.4 CONCLUSIONS

The available data for propiconazole support the proposed MOA that the induction of liver tumors in male mice is attributable to activation of CAR, which results in a series of downstream events, ultimately leading to tumorigenesis as described above and inFigure 1. The available data also demonstrate that this mode of action is not relevant for human hazard/risk assessment purposes due to qualitative differences in response to CAR activation between mice and humans. In summary, the data support the conclusion that propiconazole does not pose a hepatocarcinogenic hazard to humans. Figure 1 summarises the interspecies differences in response to propiconazole.

FIGURE 1 Summary of interspecies differences in response to propiconazole



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