CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Cyproconazole

EC Number: Not available

CAS Number: 94361-06-5

Index Number: Not available

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Version number: 1 **Date:** 2014

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Part A.

Part A.

1   PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1   Substance

Table 1:   Substance identity

Substance name:	Cyproconazole
EC number:	Not available
CAS number:	94361-06-5
Annex VI Index number:	650-032-00-X
Degree of purity:	Min. 94% w/w Cyproconazole has two diastereomeric pairs of enantiomers. (Diastereoisomer A: 430 – 500 g/kg, Diastereoisomer B: 470 – 550 g/kg).
Impurities:	Confidential Information. See Confidential Data & Information, Cyproconazole CAR/DAR. (See Technical dossier in IUCLID 5, section 1.2)

1.2   Harmonised classification and labelling proposal

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

	CLP Regulation	Directive 67/548/EEC (Dangerous Substances Directive; DSD)
Current entry in Annex VI, CLP Regulation	Acute Tox 4*; H302 Repr. 2; H361** Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Xn; R22 Repr. Cat 3; R63 N; R50/R53
Current proposal for consideration by RAC	**Carc. 2; H351 Repr. 1B; H360D STOT RE 2; H373(liver) (oral)**	**Carc Cat 3; R40 Repr Cat 2; R61 Xn; R48/22**

	Acute M-factor: 10 **Chronic M-factor: 10**	
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 4; H302 Carc. 2 H351 Repr. 1B; H360D STOT RE 2; H373 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 Acute M-factor: 10 Chronic M-factor: 10.	Xn; R22 Carc Cat 3; R40 Repr. Cat 2; R61 Xn; R48/22 R50/R53

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 1: 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	-	-	-	Conclusive but not sufficient for classification
2.2.	Flammable gases	-	-	-	Not applicable to solids
2.3.	Flammable aerosols	-	-	-	Not applicable to solids
2.4.	Oxidising gases	-	-	-	Not applicable to solids
2.5.	Gases under pressure	-	-	-	Not applicable to solids
2.6.	Flammable liquids	-	-	-	Not applicable to solids
2.7.	Flammable solids	-	-	-	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	-	-	-	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	-	-	-	Not applicable to solids
2.10.	Pyrophoric solids	-	-	-	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	-	-	-	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	-	-	-	Not applicable to solids
2.14.	Oxidising solids	-	-	-	Conclusive but not sufficient for classification
2.15.	Organic peroxides	-	-	-	Not applicable to solids
2.16.	Substance and mixtures corrosive to metals	-	-	-	Data lacking
3.1.	Acute toxicity - oral	Acute Tox. 4; H302	-	Acute Tox. 4; H302	-
	Acute toxicity - dermal	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Conclusive but not sufficient for classification

3.2.	Skin corrosion / irritation	-	-	-	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-	-	-	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Data lacking
3.4.	Skin sensitisation	-	-	-	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	-	-	-	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc. 2; H351	-	-	-
3.7.	Reproductive toxicity	Repr. 1B; H360D	-	Repr. 2; H361	-
3.8.	Specific target organ toxicity –single exposure	-	-	-	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 2; H373	-	-	
3.10.	Aspiration hazard	-	-	-	Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Acute M factor: 10 Chronic M factor: 10	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	
5.1.	Hazardous to the ozone layer				

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

Labelling: <u>Signal word:</u>   Danger

<u>Hazard statements:</u>   Acute Tox 4; H302: Harmful if swallowed
Carc. 2; H351: Suspected of causing cancer
Repr. 1B; H360D: May damage the unborn child
Stot RE 2; H373: May cause damage to organs (liver) through prolonged or repeated exposure
Aquatic Acute 1;
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects

<u>Precautionary statements:</u>   P102: Keep out of reach of children
P201: Obtain special instructions before use
P280: wear protective gloves
P301+P310: IF SWALLOWED: Immediately call a Poison Center or Doctor/Physician
P273: Avoid release to the environment
P391: Collect spillage
P501: Dispose of contents/container to…

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Cyproconazole is currently classified and included in Annex VI of Regulation (EC) 1272/2008. The substance was first discussion in 1994 and the final conclusion was reached in Nov. 1997.

2.2 Short summary of the scientific justification for the CLH proposal

Health Effects CLH proposal

Acute toxicity: The acute toxicity classification was agreed and was included in Annex of EU Dir 67/548 (26th ATP) and is now included in Annex VI of the CLP Regulation (EC) 1272/2008. This classification is supported by the data submitted under the PPP review.

Carcinogenicity: Cyproconazole is not currently classified as a carcinogen. In the dossier presented for the PPP review, carcinogenicity was investigated in both rats and mice. There were no treatment-related neoplasms observed in male or female rats at the highest dose level, thus cyproconazole is not considered to be carcinogenic in rats.

Cyproconazole was found to be carcinogenic in mice. Long-term administration of cyproconazole at doses of 100 ppm (equivalent to 13.17 mg/kg bw/day) and above caused a significant increase in the incidence of hepatocytic adenomas and carcinomas in males. In females these neoplastic changes were observed at doses of 200 ppm (equivalent to 36.30 mg/kg/day).

Supplementary investigative studies (including those using CAR-null mice) suggest a cytotoxic mode of action in mice in conjuction with effects similar to those of phenbarbitol, thus also implicating nuclear receptor (CAR/PXR) activation. The data from these supplimentary studies may not be sufficient to eliminate concern for the relevance of these effects as seen in mice and extrapolated to man. Therefore, it is proposed that classification as a Category 2 carcinogen should be further considered, but in the context of the relevance of such mouse tumours to man.

Developmental toxicity: The reproductive toxicity of cyproconazole was investigated in a 2-generation study in the rat. Developmental toxicity was investigated in the rat and rabbit. There was no effect on fertility or reproductive performance in a single 2 generation study in the rat conducted with up to 120 ppm (8-13 mg/kg bw/day) cyproconazole. Minimal parental toxicity was recorded in F0 males only in this study. There was evidence of foetotoxicity/embryotoxicity (in the form of post-implantation loss) at the highest dose.

Cyproconazole treatment resulted in significant embryo/foetal toxicity in the rat from 24 mg/kg bw/day (increased post implantation loss, reduced foetal body weight and reduced and/or delayed ossification). Cyproconazole was clearly shown to induce serious malformations at the higher doses in the rat developmental toxicity studies. Hydrocephalus and palatoschisis occurred in all three rat studies and was seen from 20 mg/kg bw/day (Machera, 1996). At dose levels where malformations occurred, maternal toxicity was recorded.

Some maternal toxicity was seen in pregnant rabbits at 50 mg/kg bw/day. In the first study, post implantation loss was increased at 10 mg/kg bw/day. This was not apparent in the second study where there was an increase in skeletal malformations at 50 mg/kg bw/day and possibly at 10 mg/kg bw/day.

Cyproconazole is currently classified for developmental toxicity as Repr. 2; H361. However, the second rabbit study (Muller, 1991 SAN 619F-Oral (Gavage) Teratogenicity Study in the rabbit, No. 252-060, Syngenta SAN619/5393) submitted as a part of the PPP review dossier, was **not** part of the data on the basis of which the 1997 classification was agreed. On revision of the whole data base and in the light of comments made by several member states and EFSA during the original peer review and including also consideration of the classification criteria of the CLP Regulation (EC 1272/2008 and the Guidance to Regulation (EC) No. 1272/2008 on Classification, Labelling and Packaging of substances and mixtures), a reproductive toxicity classification of Repr. 1B; H360d is now proposed. This is based primarily on the occurrence of malformations in both rats and rabbits at doses not causing overt signs of significant maternal toxicity.

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
Acute Tox 4; H302: Harmful if swallowed
Repr. 2; H361: Suspected of damaging the unborn child.
Aquatic Acute 1; H400: Very toxic to aquatic life.
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects.

Labelling:

Signal word:	Warning
Hazard pictogram:	GHS07
	GHS08
	GHS09

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

Classification
Xn; R22:
Repr. Cat 3; R63
N; R50/53

Labelling:

Category:	Harmful
	Dangerous for the environment
Hazard symbol:	Xn, N

R-phrases:

R22: Harmful if swallowed
R63: Possible risk of harm to the unborn child
R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S-phrases:

S2: Keep out of the reach of children
S36/37: Wear suitable protective clothing and gloves
S46: If swallowed, seek medical advice immediately and show this container or label.

2.4   Current self-classification and labelling

Not applicable.

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

3   JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Cyproconazole is a pesticide active substance currently under review for approval to Regulation (EC) No 1107/2009 of the European Parliament and of the Council. The classification and labelling proposal includes mammalian and environmental toxicity endpoints and needs to be evaluated under the CLP Regulation.

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:	Not available
EC name:	Not available
CAS number (EC inventory):	94361-06-5
CAS number:	
CAS name:	1H-1, 2, 4-triazole-1-ethanol, α-(4-chlorophenyl)-α-(1-cyclopropyl-ethyl)-
IUPAC name:	(2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol
CLP Annex VI Index number:	650-032-00-X
Molecular formula:	C15H18ClN3O
Molecular weight range:	291.8 g/mol

Structural formula:

Cyproconazole is a mixture of four stereoisomers: two diasteriomeric pairs of enantiomers, which means there are two enantiomers for each of the diasteriomers.

Diasteriomer A: enantiomeric pair, where the 3-hydroxy group and the 2-hydrogen are located on the same side (2S, 3S and 2R, 3R).

Diasteriomer B: enantiomeric pair, where the 3-hydroxy group and 2-hydrogen are located on opposite sides (2R, 3S and 2S, 3R).

Cyproconazole, 2S, 3S – enantiomer (diastereomer A)
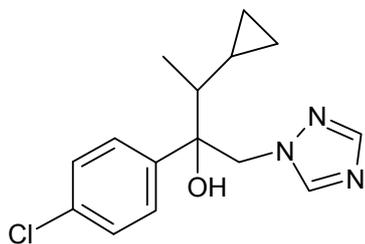
Cyproconazole, 2R, 3R – enantiomer (diastereomer A)
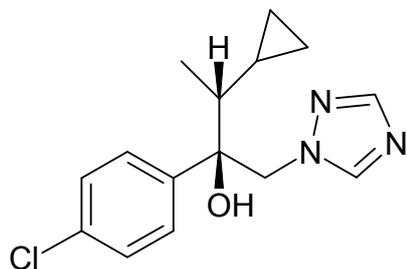
Cyproconazole, 2S, 3R – enantiomer (diastereomer B)
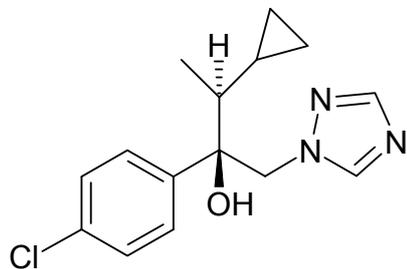
Cyproconazole, 2R, 3S – enantiomer (diastereomer B)
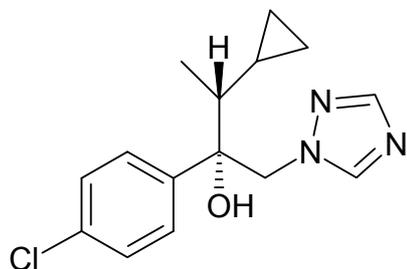
1.2   Composition of the substance

Table 5:   Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Cyproconazole	94%		-

Current Annex VI entry:

Table 6:   Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
-	-	-	-

Current Annex VI entry:

Table 7:   Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-	-	-	-	-

Current Annex VI entry:

1.2.1   Composition of test material

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	Solid	R. Das, SAN619/6781, September 1999 & R. Das, SAN619/6780, September 1999.	No comment.
Melting/freezing point	106.2 - 106.9°C ± 0.4°C	R. Das, SAN619/0447, October 1998.	Measured.

Property	Value	Reference	Comment (e.g. measured or estimated)
Boiling point	Due to the thermal decomposition of the test substance it was not possible to determine the boiling point under normal pressure (99.7%)	R. Das, SAN619/6876, April 2000.	Measured.
Relative density	Relative Density = 1.25 at 21°C	H. H. Fuldner, SAN619/0503, November 1998.	Measured.
Vapour pressure	The vapour pressure at 25°C was found by extrapolation to be 2.6 x 10^{-5} Pa.	H. Widmer, SAN619/0532, December 1998.	Estimated.
Surface tension	The surface tension was found to be 65.2 mN m^{-1} at 90% of the saturated concentration (T = 20°C).	D. Richer, N. Martin, SAN619/6767, June 1999.	Measured.
Water solubility	pH 4.1 (n = 2) a.i.: 108 ± 8 ppm pH 7.1 (n = 2) a.i.: 93 ± 18 ppm pH 10.0 (n = 2) a.i.: 109 ± 4 ppm	J. C. Karapally, M. Wilson, R. Le Discorde, SAN619/6125, March 1999.	Measured.
Partition coefficient n-octanol/water	K_{ow}= 1230.1± 61.1 (n = 6) at 25°C. $Log_{10} K_{ow}$ = 3.09.	J. Stulz, SAN619/0518, December 1998.	Measured.
Flash point	Not applicable to solids.	-	-
Flammability	A Preliminary Test was carried out using a Bunsen burner. The test substance could not be ignited (it melted) therefore the main test was unnecessary. The test substance melted, coloured yellow/brown and emitted sparks and grey smoke when in contact with the ignition source. The technical material is not flammable.	J. A. M. W. van Helvoirt, SAN619/6239, October 1994.	Measured.
Explosive properties	The Technical material was exposed to thermal	H. J. Krips, SAN619/5160,	Measured.

Property	Value	Reference	Comment (e.g. measured or estimated)
	and mechanical stress. No reaction was observed from either test.	January 1996.	
Self-ignition temperature	No exothermal effects in the temperature range 30-400°C were observed.	J. A. M. W. van Helvoirt, SAN619/6239, October 1994.	Measured.
Oxidising properties	The maximum burning rate of the test substance mixture tested was higher than the maximum burning rate of the reference mixture of barium nitrate. The results from this test indicated that cyproconazole is oxidising. However the applicant has argued that this test result is "a false positive". The burning behaviour of the cellulose/test substance mixtures differed from the burning behaviour of the tested references. The combustion of the cellulose/barium nitrate was observed at the surface of the piles and combustion of the cellulose nitrate was observed through the whole pile. Also an additional test was carried out in an inert atmosphere. It was found that test substance/cellulose mixture (60:40) could not sustain a burning reaction in an inert atmosphere. Furthermore, on consideration of the chemical structure of cyproconazole it seems evident that it would not	H. J. Krips, SAN619/6238, August 1995.	Measured.

Property	Value	Reference	Comment (e.g. measured or estimated)
	be an oxidiser.		
Granulometry	No data available	-	-
Stability in organic solvents and identity of relevant degradation products	No data available	-	-
Dissociation constant	The study concludes that cyproconazole will not dissociate in water at environmental pH.	J. C. Karapally, H. Kamp, SAN619/6138, April 1989.	Measured.
Viscosity	Not applicable to solids.	-	-

2   MANUFACTURE AND USES

2.1   Manufacture

Not relevant for this dossier

2.2   Identified uses

Wood preservative (PT 8) – Biocide

Fungicide - Plant protection

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9:   Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A.10& EEC A.16	Cyproconazole is not considered highly flammable and shows no signs of self ignition	Does not classify as being flammable.	v. Helvoirt, 1994a SAN619/6239
EEC A.14	Cyproconazole is not considered an explosive in accordance with EEC Method A.14	Does not classify as being explosive.	Krips, 1996 SAN619/5160
EEC A.17	Cyproconazole is not considered an oxidizing substance	Does not classify as being oxidizing.	Krips, 1995 SAN619/6238

3.1 RELEVANT HAZARD CLASS FOR PHYSICO-CHEMICAL PROPERTIES

3.1.1 Summary and discussion of Cyproconazole

Not applicable.   Cyproconazole does not classify with respect to its physical and chemical properties.

3.1.2 Comparison with criteria

Not applicable.

3.1.3 Conclusions on classification and labelling

Not applicable.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Mammalian toxicity studies were conducted on cyproconazole (code-named SAN 619F) and metabolite M36 which was confirmed as being present in rat urine and faeces. The purity of cyproconazole both radiolabelled and non-radiolabelled was reported to be between 91 and 99.9%. All tests were conducted in accordance with GLP. A number of studies were performed prior to the guidelines and deviated slightly, however these deviations were not considered to affect the validity of the studies or the scientific outcomes.

Absorption

Cyproconazole (radiolabelled) was found to be rapidly and extensively adsorbed at the low dose (10 mg/kg) with maximal blood levels reached between 1.5 – 7 hours post dosing. Rats administered the high dose (130 mg/kg) displayed a slower but equally extensive adsorption, with maximal blood concentrations reached between 24- 48 hours. Based on bile duct cannulated experiment cyproconazole had a total bioavailability of > 86%. Repeated administration resulted in an increased absorption with levels in the blood reaching a maximum after 8 days despite further administration. Based on the absorption studies provided there was no evidence of accumulation in any tissues of the rat.

Distribution

Cyproconazole was found to have a rapid and extensive volume of distribution as evidenced by maximal blood levels being reached between 1.5 and 7 hours and with most tissues having higher residue levels than found in the blood by 3 hours. Cyproconazole was found to be predominately associated with the organs of elimination (kidney, liver and pancreas) as well as the spleen and adrenal glands. Following repeated administration a similar pattern of distribution was observed, with cyproconazole accumulating in all tissues up to 7 days after which point no further accumulation was observed despite further dosing. Upon cessation of treatment cyproconazole was rapidly eliminated from all tissues with no signs of residue retention.

Metabolism

The metabolic profile of cyproconazole is based on the fate of [Phenyl-U-^{14}C] labelled SAN619-F, with conclusions based on both single and multiple dosing. Cyproconazole was extensively metabolised, with a greater number of metabolites identified in the urine in comparison to the faeces. In the faeces, parent cyproconazole and the identified metabolite NOA421152 are the major components and accounted for 9.5% and 2.2% respectively (after single dosing). The metabolite pattern was almost identical after multiple dosing with slight quantitative differences. Parent cyproconazole and NOA421153 were also found in the urine but less than other metabolites, however the metabolic profile in the urine was the same after single and multiple dosing. As with multiple dosing, pre-treatment with a larger dose of cyproconazole was found to have no effect on the metabolism profile. Further analysis of the metabolism profile revealed 35 metabolites of which 13 were considered significant. The metabolic pathway was elucidated based on the metabolite profile identified.

The predominant metabolic reactions of cyproconazole in the rat were:

a) oxidative elimination of the triazole ring,

b) hydroxylation of the carbon bearing the methyl group,

c) oxidation of the methyl group to the carbinol and further to the carboxylic acid and

d) reductive elimination of the carbon bearing the methyl group, yielding a benzyl alcohol which is further oxidised to the corresponding ketone (Figure 1).

Based upon an observation that diastereoisomer A of cyproconazole was metabolised faster than diastereoisomer B, a customized study was done. For both diastereoisomers, the route and rate of excretion were in the same range. Diastereoisomer A was biotransformed more extensively than diastereoisomer B; however, based upon metabolite profiles, the course of metabolism of the two diastereoisomers was shown to be similar.

There were no clear differences in metabolism in the rat, either between sexes, dosages or due to pre-treatment. Examination of species related differences revealed no difference in the metabolic pattern between rats and goats. *In vitro* investigation of metabolism of rat and mice livers revealed that mice metabolised cyproconazole at a slower rate but a similar spectra of metabolites was observed with the exception of the metabolite M9. Pre-treatment was found to slightly alter the rate of metabolism but not the profile in either rat or mouse, but pre-treatment increased the rate of metabolism of cyproconazole to a greater extent in the mouse.

Elimination

The major route of elimination of cyproconazole in the rat was predominately *via* the bile, accounting for approximately 75% in males and 59% in females. Elimination *via* the urine occurred to a greater extent in females (26.8%) than males (9.5%), which accounted for the difference in elimination in the bile. Faecal elimination accounted for less than 5% of the administered dose. The majority of the test substance was eliminated *via* the urine and bile within the first 48 hours post administration irrespective of the route of administration. However some of the test substance may be reabsorbed from the bile and excreted *via* the urine. Over 85% of cyproconazole was eliminated within 144 hours. Repeated administration had no significant effect upon the routes and rates of elimination compared to a single oral dose. The elimination from most tissues occurred rapidly, following monophasic kinetics. Biphasic elimination was observed for adrenals with a first phase half-life similar to the other tissues and a slower second phase. There was no sign of an unusual retention of cyproconazole-derived material in the rat after a single oral dose.
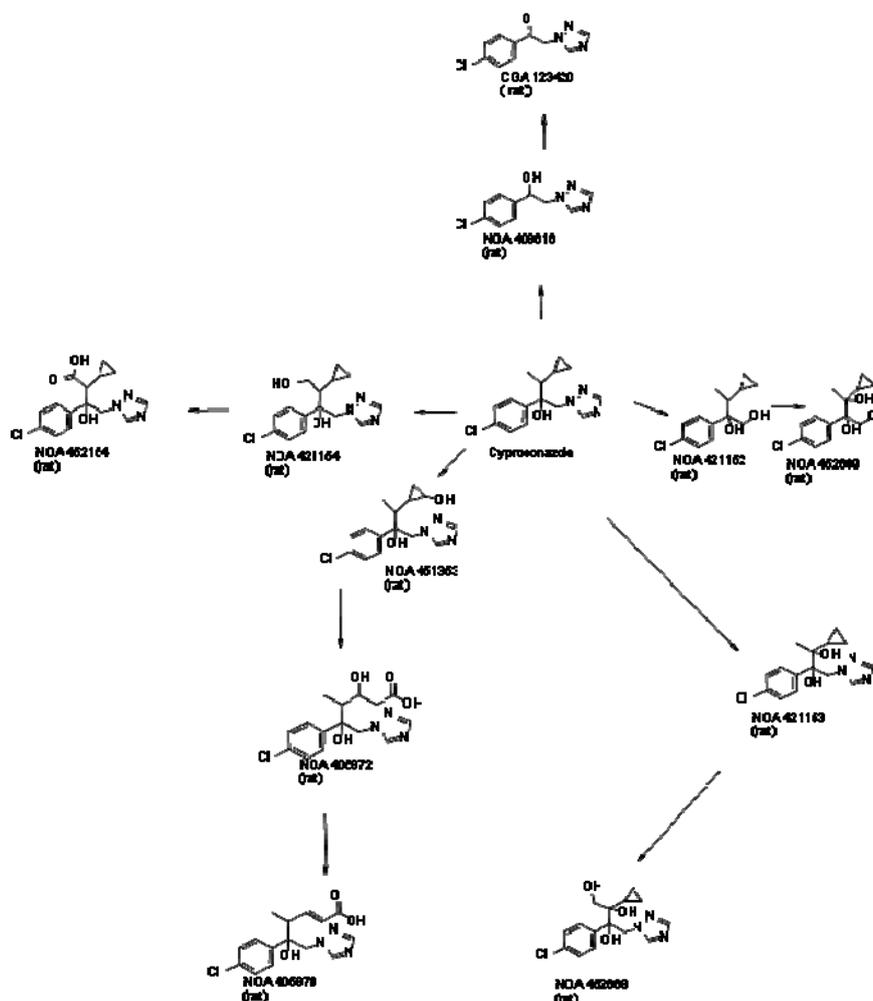
Figure 1. Summary diagram of the metabolic pathway in the rat.

4.1.2   Human information

None available.

4.1.3   Summary and discussion on toxicokinetics

Cyproconazole was found to be rapidly and extensively adsorbed with a total bioavailability of > 86%. Absorption was similar for the low (Cmax 1.5 – 7 hours after 10 mg/kg) and high doses (Cmax 24 – 48 hours at 130 mg/kg). Cyproconazole was rapidly distributed and had an extensive volume of distribution following either single or repeated administration. Residues were predominately associated with the organs of elimination (kidney, liver and pancreas) as well as the spleen and adrenal glands. Based on the absorption studies provided, there was no evidence of accumulation in any tissues of the rat.

Cyproconazole is extensively metabolised, with a greater number of metabolites identified in the urine compared to the faeces. In the faeces parent cyproconazole and the identified metabolite NOA421152 are the major components. The metabolite pattern was almost identical after multiple dosing with slight quantitative differences. Pre-treatment with a larger dose of cyproconazole was also found to have no effect on the metabolism profile. Further analysis of the metabolism profile

revealed 35 metabolites of which 13 were considered significant. Based on the metabolite profile the predominant metabolism reactions in the rat were a) oxidative elimination of the triazole ring, b) hydroxylation of the carbon bearing the methyl group, c) oxidation of the methyl group to the carbinol and further to the carboxylic acid and d) reductive elimination of the carbon bearing the methyl group, yielding a benzyl alcohol which is further oxidised to the corresponding ketone (Figure 1).

There were no clear differences in metabolism either between sexes, species (rat and goat) dosages or due to pre-treatment for the rat

Major route of elimination of cyproconazole in the rat was the bile, accounting for approximately 75% in males and 59% in females. Elimination via the urine occurred to a greater extent in females (26.8%) than males (9.5%). Faecal elimination accounted for less than 5% of the administered dose. The majority of the test substance was eliminated via the urine and bile within the first 48 hours post administration irrespective of the route of administration. However some of the test substance may be reabsorbed from the bile and excreted via the urine. Over 85% of cyproconazole was eliminated within 144 hours. Repeated administration had no significant effect upon the routes and rates of elimination compared to a single oral dose. The elimination from most tissues occurred rapidly, following monophasic kinetics.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Results		Remarks	Reference
Acute oral LD50 Han Wistar rat (male and female OECD 401 (1987)	Male: 1115 mg/kg bw Female: 1342 mg/kg bw Combined: 1290 mg/kg bw		Cat 4 H302	[Hamburger et al., 1984b] (Cyproconazole DAR Volume 3 B6.2.1.1)
*Acute oral LD_{50} Sprague Dawley rat (female) OECD 425 (2001)	Female: 350 mg/kg bw		Cat 4 H302	Durando J, 2005 *(Cyproconazole Re-registration addendum Volume 3 B.6.2.1)
Acute oral LD_{50} NMR1 mouse (male and female) OECD 401 (1987)	Male: 200 mg/kg bw Female: 218 mg/kg bw		Cat 3 H301 Because of the difference between the CLP and DSD criteria cutoff, the mouse data will trigger different classification	Hamburger et al., 1984a (Cyproconazole DAR Volume 3 B.6.2.2)
Acute oral LD_{50} CD-1 mouse (male) OECD 401 (1987)	Male: 270 mg/kg bw		Cat 3 H301 Because of the difference between the CLP and DSD criteria cutoff, the mouse data will trigger different classification.	Hamburger, 1987 (Cyproconazole DAR Volume 3 B.6.2.3)

Method	Results	Remarks	Reference
Acute oral LD$_{50}$ NZW rabbit (female) OECD 401 (1987)	Female: 460 mg/kg bw	Cat 4 H312	Hamburger et al., 1985 (Cyproconazole DAR Volume 3 B.6.2.4)
Acute inhalation LC$_{50}$ Han Wistar rat (male and female) OECD 403	greater than 5645 mg/m^3	-	Ullman L., 1985 (Cyproconazole DAR Volume 3)
Acute inhalation LC$_{50}$ *Han Wistar rat (male and female) OECD 403	greater than 2.03 mg/L	-	Durando J, 2005b (Cyproconazole Re-registration addendum Volume 3)
Acute dermal LD$_{50}$ Han Wistar rat (male and female) OECD 402	greater than 2000 mg/kg bw	-	Hamburger et al., 1984c (Cyproconazole DAR Volume 3)
Acute dermal LD$_{50}$ NZW rabbit (male and female) rabbit OECD 402	greater than 2000 mg/kg bw	-	Hamburger and Klotzsche, 1985 (Cyproconazole DAR Volume 3)
*Acute dermal LD$_{50}$ Sprague Dawley rat (male and female) OECD 402	greater than 2000 mg/kg bw	-	Durando J, 2005a (Cyproconazole Re-registration addendum Volume 3)

*New studies submitted for the PPP Review (2010).

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Data are presented for acute oral toxicity in the rat, mouse and rabbit.

Rat

Acute Oral LD$_{50}$ in Male and Female Rat. With SAN 619F; Hamburger F., Carpy S., Gerber E., Klotzsche C. November 1984: Han Wistar rats, 5 males and females were administered orally doses of 200 to 6400 mg/kg by gavage. The maximum non-lethal dose was 200 mg/kg in male and female rats. The minimum lethal dose was 320 mg/kg in females and 400 mg/kg in males. Earliest onset of lethality occurred 3.5 hours post-dose in males at 3200mg/kg and 4.5 hours in female 8000mg/kg group. The most common symptoms were signs of dazed, weakness and ataxia (females only) in both males and females at all dose levels. Less common were exophathalmus and lacrimation, also found to occur at all dose levels in both males and females. At higher dose levels animals showed signs of decreasing respiratory rate and laboured breathing. All surviving animals had showed signs of body weight gain by study termination; however animals dosed above 500 mg/kg all showed initial reduction in body weight gain over the first seven days. The LD$_{50}$ was calculated to be 1115 mg/kg for males and 1342 mg/kg for females.

*Cyproconazole Technical: Acute Oral Toxicity Up And Down Procedure In Rats with Cyproconazole Technical. Durando J, 2005: In an acute oral toxicity study, young adult female Sprague-Dawley rats were given a single oral dose of cyproconazole technical as a suspension in

1% w/w solution of carboxymethylcellulose (CMC) in distilled water, at doses of 110, 350 or 1100 mg/kg bw and observed for 14 days. An initial dose of 350 mg/kg was administered to one rat. Following the Up and Down procedure, five additional females were tested at levels of 110, 350 or 1100 mg/kg. Females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males. Two animals at 350 mg/kg and one animal at 1100 mg/kg died following signs of toxicity. At 350 mg/kg, in the two animals that died, toxic signs noted prior to death included facial staining, hypoactivity, hunched posture, an abnormal gait and/or a reduced faecal volume. The surviving animal appeared hypoactive and exhibited a reduced faecal volume, but recovered by Day 4 and appeared active and healthy for the remainder of the study. Toxic signs noted prior to death in the single animal dosed with 1100 mg/kg included hypoactivity, abnormal posture and a reduced faecal volume. One female dosed with 350 mg/kg and both animals dosed with 110 mg/kg survived. There was no effect on bodyweight gain in surviving animals. Gross necropsy of the decedent at 1100 mg/kg cyproconazole technical revealed discoloration of the intestines. The acute oral LD_{50} of cyproconazole technical was estimated to be 350 mg/kg in female rats (95% Confidence Interval of 58.05 - 1430 mg/kg).

Mouse

Acute Oral LD_{50} in Male and Female Mouse. With SAN 619F. Hamburger F, Carpy S., Gerber E, et al., October 1984: NMR1 mice, 5 males and females/group, were administered orally by gavage (10ml/kg) doses of 125 to 800 mg/kg of cyproconazole. The maximum non-lethal dose was less than 125 mg/kg in male mice and 125 mg/kg in female mice. The minimum lethal dose was 125 mg/kg in males and 160 mg/kg in females. Earliest onset of lethality occurred 14 hours after dosing in the 125 mg/kg male group and in the 800 mg/kg male and female groups. The most common symptoms were weakness, daze, decreased movement, muscle tremors, laboured and decreased respiration. The first onset of symptoms occurred within 9- 10 minutes after dosing. The longest duration of symptoms was 96 hours in the 320 mg/kg male group and 110 hours in the 500 mg/kg female group. Recovery was complete in all groups by 120 hours. The acute oral LD_{50} was calculated to be 200 mg/kg for male and 218 mg/kg for female NMR1 mice.

Acute Oral LD_{50} in Male CD1 Mice. With SAN 619F, Hamburger F., August 1987: CD-1 mice, 5 males were administered orally by gavage (10ml/kg) doses of 100 to 400 mg/kg of cyproconazole. The maximum non-lethal dose was 160 mg/kg in male mice. The minimum lethal dose was 250 mg/kg. Earliest onset of lethality occurred 39 hours after dosing in the 400 mg/kg group. The most common symptoms were weakness, dizziness, decreased movement, flaccidity, ataxia, laboured and decreased respiration. The first onset of symptoms was noted after 41 minutes post-dosing in the 400 mg/kg group. The longest duration of symptoms lasted 72 hours in the 250 mg/kg group. Recovery was complete in the survivors of all groups by 96 hours. Gross pathology did not reveal any particular findings in any organ or tissue at necropsy, except for the liver of dead animals, which were partly autolytic. The acute oral LD_{50} for SAN 619F in the male mouse is 270 mg/kg ±24.5 mg/kg.

Rabbit.

Acute Oral LD_{50} in Female NZW Rabbit. With SAN 619F. Hamburger F., Gerber E., Klotzsche C., September 1985: 5 females/group (only 2 animals at the top dose) were administered orally by gavage (5ml/kg) doses of 320 to 800 mg/kg of SAN 619F (cyproconazole). The maximum non-lethal dose was 320 mg/kg in female rabbits. The minimum lethal dose was 500 mg/kg. Earliest onset of lethality occurred within 14 hours after dosing in the 800 mg/kg group. The most common signs were weakness, dizziness and ataxia (at low dose) and at high concentrations animals displayed signs of decreased movement, flaccid and prone on one side. The earliest onset of symptoms occurred within 3 hours in the 640mg/kg group. The longest duration of symptoms was

384 hours in the 500mg/kg group. Recovery was complete in survivors of all groups by 408 hours. All surviving animals from the low dose had gained body weight at termination of the study. All other animals lost weight. Gross pathology did not show any signs of any particular findings in any organ of tissue at necropsy. The acute oral LD_{50} was determined to be 460 mg/kg for female rabbits.

4.2.1.2 Acute toxicity: inhalation

SAN619F- 4 Hour Acute Dust Aerosol Inhalation Toxicity (LC_{50}) Study with SAN619F In Rats. Ullmann L., September 1985: Han Wistar Rats (5/sex, 203-284; 10 weeks old) were exposed to test substance (2606 or 5645 mg/m^3) for 4 hours in nose-only exposure system. No animals died during the study. Slight sedation, dyspnoea and ruffled fur were observed in all animals 4 hours post dosing. All rats had recovered completely by 24 hours after initiation of exposure. Bodyweight development was not affected in males; however females showed a reduction in bodyweight gain from day1 to 8. Females had recovered by study termination and showed no treatment related effects on bodyweight. Based on the results of this study, the acute inhalation LC_{50} of cyproconazole suspended in air for male and female rats was determined to be higher than 5645 mg/m3 air (5.65mg/ L air). No classification is required.

Cyproconazole Technical: Acute Inhalation Toxicity Study in Rats with Cyproconazole Technical. Durando J, 2005b. In an acute inhalation toxicity study, five male and five female, young adult Sprague-Dawley rats were exposed by nose-only inhalation to cyproconazole technical for four hours at a concentration of 2.03 mg/L. There were no mortalities. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. All animals gained bodyweight during the study. No macroscopic findings were observed at necropsy. The acute inhalation LC_{50} for cyproconazole was estimated to be > 2.03 mg/L body weight in males and females (limit dose with no mortalities).

4.2.1.3 Acute toxicity: dermal

Acute Dermal LD_{50} in Male and Female Rats. With SAN 619F (cyproconazole). Hamburger F., Carpy S., Gerber E., Klotzsche C., November 1984: Cyproconazole (2000mg/kg) was applied for 24 hours at a dosing volume of 4ml/kg (50% solution of DMSO) to shorn skin (10% body area) of 5/sex Wistar Rats. The treated area was covered by porous gauze. No mortality occurred following dermal application of 2000mg/kg cyproconazole for 24 hours. No signs of toxicity were observed. Body weight gain was retarded in males during the first week, whereas females lost weight over the course of the study. Body weight for both sexes partially recovered during the second week. Necropsy revealed no abnormal changes. All animals showed signs of weakness within the first 2 hours post-dosing but had recovered 48 hours post-dosing. The acute dermal LD_{50} was higher than 2000mg/kg in both male and female rats.

Acute Dermal LD_{50} in Male and Female Rabbit SAN 619F. Hamburger F., Klotzsche C., July 1985: Cyproconazole (2000mg/kg) was applied for 24 hours at a dosing volume of 3ml/kg (0.9% solution of NaCl) to shorn skin (approx 10% body area) of 5 male and 5 female New Zealand White Rabbits. The treated area was covered by porous gauze. No mortality occurred following dermal application of 2000mg/kg cyproconazole for 24 hours. No symptoms of toxicity were observed. Body weight gain was reduced in males up to day 3 post-dosing, whereas females lost weight over the course of the first 4 days. Body weight for both sexes partially recovered during the remainder of the study. Necropsy revealed no abnormal changes and there were no signs of toxicity. The acute dermal LD_{50} was higher than 2000mg/kg in both male and female rabbits.

Cyproconazole Technical: Acute Dermal Toxicity Study in Rats – Limit Test with Cyproconazole Technical. Durando J, 2005a. In an acute dermal toxicity study, young adult Sprague-Dawley rats (5 male and 5 female) were dermally exposed to cyproconazole technical (98.2% a.i) as a dry paste (70% w/w mixture in distilled water) for a single 24-hour application to approximately 10% of the body surface at a limit dose of 2000 mg/kg bw. There were no mortalities. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects or abnormal behaviour. All animals gained bodyweight during the study. No macroscopic findings were observed at necropsy. The acute dermal LD_{50} for cyproconazole was estimated to be > 2000 g/kg body weight (limit dose with no mortalities).

4.2.1.4 Acute toxicity: other routes

No data.

4.2.2 Human information

No data.

4.2.3 Summary and discussion of acute toxicity

The acute oral toxicity of cyproconazole was tested in the rat, mouse and rabbit. The test substance was found to be harmful in both the rat and rabbit and toxic to the mouse. There were no adverse effects *via* the dermal and inhalation routes. A new acute toxicity data set was reviewed as part of the re-submission. The compound can be considered to be Acute Tox 4 H302 based on the most recent acute oral study in the rat which is the preferred test species for this end-point. There was no evidence of significant toxicity in the rat by either the inhalation or dermal route.

4.2.4 Comparison with criteria

Oral

Taking the rat oral data, (LD_{50} 350 mg/kg bw/day) cyproconazole will classify as H302: Harmful if swallowed using the CLP Regulation (> 300 ≤ 2000). If the mouse data are considered relevant (LD_{50} 200 mg/kg /day), then the classification becomes Cat 3 H301: 'Toxic if swallowed' according to the CLP (> 50 ≤300).

While in this case the mouse was the most sensitive species, the rat is (generally) the preferred species for acute oral toxicity classification. In the rat studies the LD_{50} ranges from 1115 down to 350 mg/kg bw/day; in the rabbit the oral LD_{50} is 460 mg/kg bw/day. One mouse study gave an oral LD_{50} of 270 mg/kg bw/day (H301) and in the second mouse study; the male LD_{50} is 200 mg/kg bw/day and the female 218 mg/kg bw/day.

4.2.5 Conclusions on classification and labelling

Considering the weight of evidence, a classification of Acute Tox 4; H302 is considered more reflective of the data. The current listed oral toxicity classification (Annex VI) appears to be based on the same data set as described above but without the new rat data submitted for the PPP Re-review (marked * in Table 10 above). These studies further support Acute Tox 4 H302.

CLP: Acute Tox. 4 - H302.

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

There is no indication from the data presented that specific target organ toxicity will result from a single exposure. Cyproconazole does not need to be classified for specific target organ toxicity.

4.4   Irritation

4.4.1   Skin irritation

Table 11:   Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation in NZW rabbits. Cyproconazole (94.4% purity) OECD 404	Non-irritating	-	Hamburger, F. et al., 1985a (Cyproconazole DAR Volume 3 B.6.2.8)
Skin irritation in NZW rabbits. Cyproconazole (98.2% purity) OECD 404	Non-irritating	-	Durando J, 2005c. (Cyproconazole Re-registration addendum Volume 3 B.6.2.4.1)

4.4.1.1 Non-human information

SAN619F-Primary Skin Irritation In Rabbits. Hamburger F, Gerber E, Klotzsche C. March 1985. In a primary dermal irritation study, young adult (3 male) New Zealand albino rabbits were dermally exposed to 0.5 g of cyproconazole technical (94.4% a.i dissolved in DMSO 50%) for a single 4-hour application to one intact site on each animal. Tested animals displayed no signs of either erythema or oedema at any point throughout the course of the study.

Cyproconazole Technical: Primary Skin Irritation Study In Rabbits with Cyproconazole Technical. Durando J, 2005c. In a primary dermal irritation study, young adult (2 male and 1 female) New Zealand albino rabbits were dermally exposed to 0.5 g of cyproconazole technical (98.2% a.i.) as a dry paste (70% w/w mixture in distilled water) for a single 4-hour application to one intact site on each animal. One hour after patch removal, very slight erythema was noted for all three treated dose sites. All animals were free from dermal irritation within 24 hours.

4.4.1.2 Human information

Not available.

4.4.1.3 Summary and discussion of skin irritation

Cyproconazole was not irritating to the skin in the studies presented.

4.4.1.4 Comparison with criteria

Not relevant.

4.4.1.5 Conclusions on classification and labelling

CLP: No classification based on available data.

4.4.2   Eye irritation

Table 12:   Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation in NZW rabbits (94.4% purity) OECD 405	Non-irritant	Slight reversible erythema.	Hamburger, F. et al., 1985b (Cyproconazole DAR Volume 3 B.6.2.9)
Eye irritation in NZW rabbits (98.2% purity) OECD 405	No classification	Slight to moderate erythema reversible within 72 hours.	Durando J, 2005d (Cyproconazole Re-registration addendum Volume 3 B.6.2.5)

4.4.2.1 Non-human information

SAN619F-Primary Eye Irritation Test in Rabbits. Hamburger F, Gerber E, Klotzsche C., March 1985. Neat cyproconazole powder (Purity 94.4%) (0.1 g) was instilled into the conjunctival sac of the right eye of three New Zealand White rabbits. The lids were thereafter gently held together for one second and then released. The left eyes served as controls. Eyes were not washed and were subsequently examined at 0.5, 24, 48 and 72 hours post dosing. Slight redness (grade 1) of the conjunctivae was noted at 0.5 h after treatment in all animals tested. No other findings were noted at all thereafter.

Cyproconazole Technical: Primary eye Irritation Study In Rabbits with Cyproconazole Technical. Durando J, 2005d.   In a primary eye irritation study, 0.1 mL (0.06 g) of finely ground cyproconazole technical (98.2 % a.i.) was instilled into the conjunctival sac of the right eye of each of three, young adult, New Zealand albino rabbits (1 male and 2 females). The other eye of each rabbit remained untreated with the test substance and served as a control. The treated eyes were not rinsed after instillation.

No deaths occurred. No systemic signs of toxicity were noted during the study. Within one hour after test substance instillation, iritis grade 1 and conjunctivitis grade 2 were noted for all three treated eyes. The iritis had regressed by 24 hours. Conjunctival reaction regressed to grade 1 by 24 hours and 0 at 48 hours. All animals were free of ocular irritation within 72 hours.

No abnormal findings were observed in the treated eye of any animal 72 hours after treatment. Under the conditions of this study, cyproconazole technical caused mild to moderate transient irritation to the eye. All reactions were resolved by 72 hours.

Table 13: Primary eye irritation scores (Durando, 2005d)

Time	Cornea			Iris			Conjunctiva					
							Redness			Chemosis		
Animal number	**5**	**6**	**7**	**5**	**6**	**7**	**5**	**6**	**7**	**5**	**6**	**7**
after 1 hour	0	0	0	1	1	1	2	2	2	1	1	1
after 24 hours	0	0	0	0	0	0	1	1	1	0	0	0
after 48 hours	0	0	0	0	0	0	1	1	0	0	0	0
after 72 hours	0	0	0	0	0	0	0	0	0	0	0	0
mean scores 24-72h	0	0	0	0	0	0	0.7	0.7	0.3	0	0	0

4.4.2.2 Human information

Not available

4.4.2.3 Summary and discussion of eye irritation

Slight reversible erythema was seen in the two studies presented. This was fully reversible within 72 hours.

4.4.2.4 Comparison with criteria

The individual and group mean eye irritation scores do not meet the criteria for classification as irritating to the eyes according to CLP, (corneal opacity or iritis score ≥ 1 or conjunctival redness or edema score ≥ 2) and which fully reverses within the observation period of 21 days).

4.4.2.5 Conclusions on classification and labelling

CLP: No classification based on available data.

4.4.3   Respiratory tract irritation

4.4.3.1 Non-human information

Cyproconazole is not classifiable for skin or eye irritation and respiratory tract irritation is not anticipated. There was no evidence of respiratory tract irritation in the data from the acute and subacute studies.

4.4.3.2 Human information

Not available.

4.4.3.3 Summary and discussion of respiratory tract irritation

Not indicated.

4.4.3.4 Comparison with criteria

Not relevant.

4.4.3.5 Conclusions on classification and labelling

CLP: No classification based on available data.

4.5 Corrosivity

4.5.1 Non-human information

Cyproconazole is not irritant to skin or eyes in the rat.

4.5.2 Human information

Not available.

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

Not relevant.

4.5.5 Conclusions on classification and labelling

CLP: No classification based on available data.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 14:   **Summary table of relevant skin sensitisation studies**

Method	Results	Remarks	Reference
-Magnusson and Kligman (OECD 406). -Cyproconazole (95.6% purity) -GOHI strain Guinea pigs (Himalayan Spotted)	No sensitization reaction. 24 and 48 hours after challenge application, no positive reactions were evident at the application site in any of the animals.	GLP study and checked for compliance with the OECD 406 (1992) guideline.	Arcelin G., 1992. (Cyproconazole DAR Volume 3 B.6.2.10)

Method	Results	Remarks	Reference
Beuhler Method. (OECD 406). Hartley guinea pigs	No sensitization reaction.	GLP and OECD 406 (1992) guideline study.	Durando J, 2005e (Cyproconazole Re-registration addendum Volume 3 B.6.2.6.1)

4.6.1.1 Non-human information

SAN619F-Skin Sensitisation Test in Guinea Pig. Arcelin G, January 1992: Epidermal induction (cyproconazole 5%) caused grade 1 erythema in 8 of 20 animals and in 2 of 20 animals after 24 and 48 hours, respectively. A positive control group with 2-mercaptobenzothiazol is included for sensitivity check of the Guinea pig strain. No positive reactions were evident at the application site in any of the animals at 24 and 48 hours after challenge (cyproconazole 25%) application. No toxic symptoms were evident in the Guinea pigs of the control or test group..

Cyproconazole Technical: Dermal Sensitisation Study in Guinea pigs with Cyproconazole Technical. Durando J, 2005e. In a dermal sensitisation study with cyproconazole technical (98.2%), guinea pigs were tested using the method of Buehler. The induction dose was a 75% w/w mixture of the test substance in a 1% w/w solution of CMC in distilled water. Twenty-seven days after the first induction dose, 0.4 g of a 75% w/w mixture of the test substance in a 1% w/w solution of CMC in distilled water (HNIC) was applied to each test and naive control animal as a challenge dose. a-hexyl cinnamic aldehyde (HCA) was the positive control test substance for this laboratory. Very faint erythema (0.5) was noted for several test sites throughout the induction phase. Following challenge, very faint erythema (0.5) was noted for five of twenty test sites after 24 hours. Irritation cleared from all affected sites by 48 hours.

4.6.1.2 Human information

No data available.

4.6.1.3 Summary and discussion of skin sensitisation

Dermal sensitisation was investigated in two acceptable tests, using the M&K assay and the Beuhler test. There was no response consistent with dermal sensitisation.

4.6.1.4 Comparison with criteria

Not relevant in this case.

4.6.1.5 Conclusions on classification and labelling

No sensitisation effects were detected in either a Maximisation test according to Magnusson and Kligman or the Buehler test and therefore no classification is necessary.

CLP: No classification based on available data.

4.6.2 Respiratory sensitisation

No data on respiratory sensitisation is available.

4.7 Repeated dose toxicity

Table 15: Summary table of relevant repeated dose toxicity studies

Species/study/dose	Findings at LOAEL	GV (extrap.) CLP	Reference
Rat 28-day, feeding OECD 407 (1995) Cyproconazole purity 95.7% 10, 30, 100, 300, 1000 ppm (equiv. 0.8/0.93, 2.29/2.94, 8.14/9.81, 25.32/31.54, 96.18/127.59 mg/kg d in males/females)	1000 ppm (96.18/127.59 mg/kg): Haematology Significant/severe hepatotoxicity LOAEL: 300 ppm (25.32/31.54 mg/kg bw/d) -Reduced bw gain, -significant hepatotoxicity NOAEL: 100 ppm = 8.1 mg/kg	≤300 Classify	Skinner C, Luginbühl H, Carpy S *et al.,* 1984 DAR Vol 3 B.6.3.1)
Rat 1st 90-day, feeding, OECD 408 (1998) 20, 80, 320 ppm (equiv 1.5/1.9, 6.4/7.0, 23.8/31.1 mg/kg bw in males/females)	LOAEL: 320 ppm (23.8/31.1 mg/kg bw/day) -Reduced bw gain, -haematology - hepatotoxicity reversible after recovery period, -disturbed Ca and creatinine levels NOAEL: 80 ppm = 6.4 mg/kg	≤100 No	Skinner C, Luginbühl H, Carpy S., 1985 (DAR Vol 3 B.6.3.2.1)
Rat 2nd 90-day, feeding, OECD 408 (1998) 20, 350, 700, 1400 ppm equiv 1.4/1.6, 24.7/29.6, 52.8/57.3, 107/118 mg kg bw/d, males/females)	1400 ppm (107/118 mg/kg): -significant haematology -significant hepatotoxicity 700 ppm (52.8/57.3 mg/kg) -significant haematology -significant hepatotoxicity LOAEL: 350 ppm (24.7/29.6 mg/kg bw/d) -Reduced bw gain, - hepatotoxicity, -disturbed lipid metabolism NOAEL: 20 ppm = 1.4 mg/kg	≤100 Classify	Gerspach R., 1999. (DAR Vol 3, B.6.3.2.2)
Mouse 13-Week Dose Range Finding Feeding Study In CD-1 Mice ≈OECD 408 (1998) 5, 15, 300, 600 ppm equiv 0.7/1, 2.2/3.2, 43.8/70.2, 88.8/128.2 mg kg bw/d, males/females)	LOAEL: 300 ppm (43.8/70.2 mg/kg bw/d) -Reduced bw gain, - significant hepatotoxicity NOAEL: 15 ppm = 2.2 mg/kg	≤100 Classify	Warren S, Skinner C., Karapally J., 1987 (DAR Vol 3 B.6.3.2.3)
Dog: 13-Week Feeding Study In Beagle Dogs ≈OECD 452 (1981) 20, 100, 500 ppm equiv 0.77/0.7, 4/3.25, 18.18/19.17 mg kg bw/d, males/females)	LOAEL: 500 ppm (18.18/19.17 mg/kg bw/d) -significant Hepatotoxicity, -disturbed lipid metabolism NOAEL: 100 ppm = 3.3 mg/kg	≤100 Classify	Warren, S., Skinner, C., Carpy, S., 1986 (DAR Vol 3 B.6.3.2.4)

Species/study/dose	Findings at LOAEL	GV (extrap.) CLP	Reference
Dog: Chronic Oral Toxicity By Dietary Administration To Beagle Dogs For One Year OECD 452 (1981) 30, 100, 350 ppm equiv 0.99, 3.15/3.23, 12.05/12.58 mg kg bw/d, males/females)	LOAEL: 350 ppm (12.05/12.58 mg/kg bw/d) -Hepatotoxicity, -disturbed lipid metabolism NOAEL: 100 ppm = 3.2 mg/kg	≤12.5 Classify	Warren S, Hamburger F, Carpy S *et al.,* 1988 (Amended report 1992) (DAR Vol 3 B.6.3.2.5)
Rat: Subacute (16-day) Repeated Dose Inhalation Toxicity Study In Rats OECD 412 (1981) 0.017, 0.099, 1.026 mg/L air	LOAEL: 0.099 mg/L -Hepatocellular hypertrophy NOAEL: 0.017 mg/L ai = 4.9 mg/kg	≤0.6	Bernstein D, Luetkemeier H, Vogel O, *et al.* 1987 (DAR Vol 3 B.6.3.3.2)
Rat: 28-day Repeated Dose Dermal Toxicity Study In Rats OECD 410 (1992) 10, 100, 1000 mg/kg bw	LOAEL: 100 mg/kg -Local effects at skin application site; -clinical chemistry, -haematology, - histopathology. NOAEL: 10 mg/kg bw/day	≤600	Sommer E., 2000 (DAR Vol 3 B.6.3.3.1)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Study 1. 28-day rat feeding study (Skinner et al., 1984) DAR Vol 3 B.6.3.1.1

Table 16: Summary of main findings

Study	Main findings
Rat 28-day, feeding. Cyproconazole purity 95.7% 10, 30, 100, 300, 1000 ppm (equiv. 0.8/0.93, 2.29/2.94, 8.14/9.81, 25.32/31.54, 96.18/127.59 mg/kg mg/kg/d in males/females)	**1000 ppm (96.18/127.59 mg/kg)** Mortality/clinical signs: No unscheduled deaths or treatment-related signs. Body weight and food consumption: significant ↓↓weight gain in males (-89%, -22%, -27% at wks 1, 2, 3) and females (-155%, -12% at wks 1, 2). Food consumption not affected. Haematology: significant ↑WBCs (+35%**) in males and (+26%) in females (ns). Clinical chemistry: ↑total protein wk 2 (+11%**) in females. ↑BUN wk 2 males and females (+22%* and +25%*); wk 4 males (+28%*). ↑Cholesterol wk 2 females (+77%**) and wk 4 (+70%**), slight in males at wk 4. ↓bilirubin wk 4 males (-15%, ns) and females (-44%**). ↑ (stat sig) ALAT males wk 2 (+74%**) and wk 4 (+49%**) and wk 4 females (+27%*) ↑ASAT (SGOT) ↑ wk 2 males (+36**) ↑LDH wk 2 and 4 males (* and **) and females (** and ns) Urinalysis: ↑amorphous urate wk 4 males (+200%**) and wk 2 females ((+38% ns). Organ weights: ↑ abs/rel liver wt in males (+41%**/+62%**) ↑ abs/rel liver wt in females (+35%**/+45%**) Pathology *Macroscopic:* increased liver architectural visibility, white points and hyperanemia *Microscopic:* Hepatocellular vacuoles were present in a mild or moderate degree in a majority of

	high dose males
	Hepatocytomegaly, predominantly centrilobular was recorded in 10/16 high dose males and 2/16 high dose females.
	300 ppm (25.32/31.54mg/kg/day)
	Body weight and food consumption:
	↓body weight gain wks 1 (-37%) males and wk 1 (-52%) and 2 (-1%) females.
	Organ weight
	↑ abs/rel (+16%**) liver wt in males
	↑ abs/rel liver wt in females (+15%/+18%**)
	Pathology:
	Hepatocellular vacuoles
	Clinical chemistry
	↑cholesterol in females (ns)
	↓bilirubin wk 4 males (-11% ns) and females -26% ns)
	↑LDH wk 2 (ns) and wk 4 (*) males and wk 2 (**) and wk 4 (ns) females.
	100 ppm (2.29/2.94 mg/kg)
	↓body weight gain wk 1 and 2 females (-18% and -11% ns)
	↓bilirubin wk 4 males (-12% ns)

*P<0.05, ** p<0.01, ns- not significant.

Conclusion:

A reduction in body weight gain at 8.14 mg/kg (100 ppm) in females at week 2 was considered to be of questionable biological significance as no other toxicologically significant findings were associated with this effect. Decreases in body weight gain at 25.32 mg/kg (300 ppm) and above both in males and females over the first two weeks were considered to be treatment related. These changes in bodyweight gain did not correspond to reductions in food consumption. The NOAEL was set at 8.1 mg/kg bw (100 ppm) based on these changes in body weight gain together with evidence of hepatotoxicity including significantly increased liver weight and some histopathology and increased LDH levels at 300 ppm (25.32 mg/kg bw). Therefore the LOAEL was 25.3 (300 ppm). The highest dose tested (96.18 mg/kg) can be considered an MTD, causing significant body weight and liver weight effects. Clinical chemistry and histopathology findings identify the liver as the main target organ.

Study 2. 90-day in the rat (Skinner, 1985) DAR Vol 3 B.6.3.2.1.

Table 17:   Summary of main findings

Study	Main findings	Cut-off
Rat 1st 90-day, feeding, Cyproconazole 95.7% 20, 80, 320 ppm (equiv 1.5/1.9, 6.4/7.0, 23.8/31.1 mg/kg d males/females)	**320 ppm (23.8/31.1 mg/kg bw/day)** Body weight and food consumption: ↓body weight gain in males (-18%) and females (-12.5%). Weight gain was ↓ by 24.6% during the last 5 weeks in females. Haematology: ↓Haematocrit wks 4-8 males (**) ↑MCHC wks 4-8 males (**) Clinical chemistry: ↑creatinine wks 8 (**) and 13 (*) in males and wks 4 , 8, and 13 in females (**). ↓bilirubin wks 4-8 in males (ns) and females (*) ↑LDH in males and ↓females Organ weight: ↑abs (*) and rel (**) liver weight in females and ↑ rel liver weight in males (**). Reversible during the recovery period. Histopathology: Vacuolated hepatocytes with single large or several small vacuoles predominantly centrilobular were present in 6 of 15 males treated at 320 ppm Distinct lobular pattern of liver (5 males and 4 females). Reversible within the recovery period. **≥80 ppm (6.4/7.0 mg/kg bw day)** Inconsistent fluctuations in serum Ca/Na/K	<100 mg/kg bw

*P<0.05, ** p<0.01, ns- not significant.

Conclusion:

Cyproconazole administered at 320 ppm caused slight impairment of body weight gain, increased liver weights and histopathological liver changes in male and female rats. Disturbances in clinical chemistry parameters were inconsistent, not showing any dose related pattern and fluctuated considerably over the study period. The absence of a clear dose dependency and the absence of an obvious effect at 80 ppm in most cases or concurrent histopathological changes at the same levels indicates that the findings at 20 ppm are rather due to fluctuations than due to a real treatment related effect. It is therefore concluded that the dietary dose level of 80 ppm (6.4/7.0 mg/kg) represents a NOAEL in this study. Effects at 80 ppm were of minimal degree, usually without achieving statistical significance and of dubious toxicological relevance. It is concluded, that there were no adverse effects in this study due to treatment with cyproconazole at the dose of 80 ppm. Therefore, this dose level represents a NOAEL in this study, which corresponds to mean daily intakes of 6.4 and 7.0 mg/kg bw in males and females, respectively. Subsequently, the LOAEL for this study is 320 ppm (23.8 and 31.1 mg/kg bw for males and females, respectively).

Study 3. 90-day in the rat (Gerspach, 1999) DAR Vol 3 B.6.3.2.2.

Table 18:   Summary of main findings

Study	Findings
Rat 2nd 90-day, feeding, Cyproconazole 95.5% 20, 350, 700 and 1400 ppm (equiv 1.4/1.64, 24.7/29.6, 52.8/57.3,	**1400 ppm (107/118 mg/kg bw/day)** Body weight and food consumption: ↓body weight gain in males (-28%) and females (-17%). ↓terminal body weight (-19% and -10%) for males and females respectively. ↓food consumption wk 1 by 16% and 6% (males and females), Haematology:

| 107/118 mg/kg d males/females) | ↑ in RBC, RDW and HDW (p<0.01)
↓ Hb, MCV, RDW and MCH (p<0.01)
↑ prothrombin time (p<0.01)
-slight leucocytosis in males and females (p<0.01)
Clinical chemistry:
↑urea, protein, globulin, cholesterol, Na, K, Ca,
ALAT, ASAT and GGT in males and females (also AP), p<0.01.
↓bilirubin, albumin:globulin ratio, triglycerides, Cl, in males and females
Organ weight:
↓carcass weight (-19% and -8%)
↑abs/rel liver weight (+25%/+55%) in males (+34%/+47%) in females
↑ rel adrenal weight in females (sig. trend)
↓abs/rel spleen (-28%/-21%) in females (sig. trend)
Histopathology:
(see table below).

≥700 ppm (52.8/57.3 mg/kg bw day)
Body weight and food consumption:
↓body weight gain 1st 4 wks in males (-25%) and females (-12.6%)
↓terminal body weight (-10%) and -5%) for males and females respectively.
↓food consumption by 6% in males.
Haematology:
↑ in RBC, RDW and HDW (p<0.01)
↓ Hb, MCV, RDW and MCH (p<0.01)
↑ prothrombin time (p<0.01)
-slight leucocytosis in males and females (p<0.01)
Clinical chemistry
↑urea, protein, globulin, cholesterol, Na, K, Ca,
ALAT, ASAT and GGT in males and females, p<0.01.
↓bilirubin, albumin:globulin ratio, triglycerides, Cl, in males and females
Organ weight:
↓carcass weight in males (-9%)
↑abs/rel liver weight (+14%/+25%) in males (+28%/+31%) in females
↑ rel adrenal weight in females (sig. trend)
↓abs/rel spleen (-27%/-25%) in females (sig. trend)
Histopathology:
(see table below).

350 ppm (24.7/29.6 mg/kg bw/day)
Body weight and food consumption:
↓body weight gain 1st 4 wks in males (-2.5%) and females (-1.4%)
↓terminal body weight (-5%) for females
↓food consumption (not stat. sig.)
Haematology:
↑ prothrombin time in females (p<0.01)
Clinical chemistry
↑urea, protein, globulin, cholesterol, Na, K, Ca,
in males and females, p<0.01.
Organ weight
↑abs/rel liver weight (males +14%/+13%; females +17%/+18%)
↑ rel adrenal weight in females (sig. trend)
Histopathology:
(see table below) |

Table 19: **Summary of histopathological findings**

	Males					Females				
Dose level (ppm)	**0**	**20**	**350**	**700**	**1400**	**0**	**20**	**350**	**700**	**1400**
No. of tissues examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal glands (cortex)										
Fatty change (average grading)	5 (1.0)	3 (1.0)	6 (1.2)	10 (1.4)	10 (1.3)			2 (1.0)	2 (1.0)	1 (1.0)
Deposition of ceroid (average grading)						1 (1.0)	2 (1.0)	1 (2.0)	7 (1.6)	9 (2.1)
Single cell necrosis (average grading)					1 (1.0)			1 (1.0)	2 (2.0)	10 (2.0)
Kidneys										
Haemosiderosis (average grading)					4 (1.3)				7 (2.6)	10 (3.5)
Liver										
Fatty change (average grading)			6 (1.8)	9 (2.3)	9 (2.6)					
Hepatocellular hypertrophy (average grading)			10 (2.1)	10 (3.0)	10 (3.9)			7 (1.3)	7 (1.1)	10 (1.9)
Pituitary gland										
Hypertrophy, distal lobe (average grading)	4 (1.3)	5 (1.2)	9 (1.7)	10 (2.0)	8 (1.9)		1 (1.0)	1 (1.0)		
Spleen										
Extramed. haematopoiesis (average grading)	10 (2.7)	10 (2.7)	10 (2.7)	8 (1.9)	1 (3.0)	10 (3.0)	10 (3.0)	10 (1.7)	10 (1.2)	7 (1.0)
Haemosiderosis (average grading)	10 (2.0)	10 (1.9)	10 (3.1)	10 (4.3)	10 (2.9)	10 (3.1)	10 (3.5)	10 (4.2)	10 (3.7)	10 (2.4)
Thyroid gland										
Follicular hypertrophy (average grading)		5 (1.2)	4 (1.8)	10 (2.2)	9 (1.8)		1 (1.0)	3 (1.3)	7 (1.4)	10 (1.6)

The following treatment related changes were found: adrenal glands had an increased incidence and grading of cortical fatty change in males and females treated at 350 ppm, 700 ppm and 1400 ppm. Deposition of ceroid occurred with increased incidence/grading in female of the three higher dose groups. Necrosis of single cortical cells, mainly of those located at the border of fascicular and reticular zones, was observed in high dose males and female treated at 350 ppm and higher.

The kidneys showed some signs of atrophy at the lower doses and haemosiderosis in high dose males and female treated at 700 and 1400 ppm. 100% of the female animals displayed widespread and moderate mineralisation in the kidneys, with an unidentified cause.

In the liver, fatty change and hypertrophy of hepatocytes (centrilobular zones) occurred in males of the dose groups 350, 700 and 1400 ppm. Hepatocellular hypertrophy was also observed in females of the same groups.

The pituitary gland had an increased incidence/grading of cellular hypertrophy in the distal lobe of the three higher dose groups of males. The cells with large, pink cytoplasm and occasional cytoplasmic vacuoles were probably thyrotrophs.

The spleen exhibited a suppression of incidence/grading of extramedullary haematopoiesis in the two high dose male groups and in female of the three higher dose groups. Haemosiderosis had increased grading in males dosed at $\geq$350 ppm and in females dosed at $\geq$700 ppm.

The thyroid gland showed increasing incidence/grading for hypertrophy of follicular cells in all treated male and female groups. However, the incidence and grading of the findings in low dose males and females of the present study were comparable to those occasionally occurring in the untreated control groups observed in the laboratory in the same kind of studies and the same strain of rats. A peer review of control animals from five studies performed between December 1996 and June 1997 revealed the following results:

Study	Males					Females				
	A	B	C	D	E	A	B	C	D	E
No. of tissues examined	10	10	10	10	10	10	10	10	10	10
Thyroid gland										
Follicular hypertrophy (average grading)	2 (1.5)	0	4 (1.8)	6 (2.2)	2 (1.5)	0	2 (2.5)	0	2 (2.0)	0

It is evident that incidence and grading were higher in some of the control groups from other studies. Therefore, although the values in a number of groups are above the values of the concurrent male and female control groups, they are not considered to represent a treatment related effect.

Conclusion

Decreased body weight gain was observed at all dose levels in both males and females over the first 4 weeks of the study. The effects at 350 ppm and above are considered to be of toxicological significance. Enzyme induction and metabolic adaptations are possibly secondary to effects on the thyroid/pituitary axis, which appears evident in males. In addition, an adrenocorticotropic effect was indicated, due to the histopathological changes in the pituitary and adrenal glands as well as some evidence from altered electrolytes in the blood. Based on the results of this study the starting dose of 20 ppm is considered too low as the next dose level (350 ppm) was very much higher and showed toxicologically relevent adverse effects.

Study 4. 90-day study in mice (Warren, S., 1987) DAR Vol 3 B.6.3.2.3.

This study had methodological limitations in that no haematology or clinical chemistry parameters were investigated. Spacing between the doses is considered to too large to enable any conclusions in relation to the NOAEL.

Table 20:   Summary of study findings

Study	Findings
Mouse 90-day, feeding, Cyproconazole 95.6% 5, 15, 300, 600 ppm (equiv 0.7/1.00, 2.2/3.2, 43.8/70.2, 88.8/128.2 mg/kg d males/females)	**600 ppm (88.8/128.2mg/kg bw/day)** Body weight and food consumption: ↓body weight gain (wks 0-13) in males (-68%) and females (-35%). ↓terminal body weight (-11% and -7.4%) for males and females respectively. ↓food consumption wk 1 (males and females), Organ weight: ↓carcass weight (-19% and -8%) ↑abs/rel liver weight (**) in males and in females ↓rel spleen (+50%) in males and (+22%) in females (**) ↓ abs. kidney weight (>10%) males and females. Pathology: ↑ accentuated hepatic lobular pattern in males and females

	See Table **≥300 ppm (43.8/70.2 mg/kg bw day)** Body weight and food consumption: ↓body weight gain wks 0-13 in males (-43%) and (-22%) in females. ↓terminal body weight (-9.5%) for males. ↓food consumption in males. Organ weight: ↓carcass weight in males (-9%) ↑abs/rel liver weight (**) in males (**) in females ↓rel spleen (+48%) in males Histopathology: (see below). **15 ppm (2.2/3.2 mg/kg bw/day)** Body weight and food consumption: ↓body weight gain in males (-13%) and females (-19%) Organ weight ↑abs/rel liver weight (males +14%/+13%; females +17%/+18%) ↑ rel adrenal weight in females (sig. trend) **5 ppm. (0.7/1.00 mg/kg bw/day)** ↓body weight gains (males)

Histopathology:

Treatment-related effects were present only in hepatocytes. A statistically significant increased incidence of hepatocyte change in males treated at 300 ppm, or females treated at 600 ppm, was noted. Hepatocyte changes in the higher dosage groups included vacuolation of hepatocytes in the periacinar region, more marked and more frequent among female mice; single cell necrosis in both sexes; and eosinophilia of hepatocytes in the periacinar region more marked and more frequent in males. These histopathological changes are clear indications of toxicity due to exposure to cyproconazole.

Table 21:   Histopathological finding associated to treatment with SAN619

Findings	Group	1	2	15	300	600
Periacinar hepathocytic vacuolisation	Male	0/10	0/10	0/10	0/10	4/10
	Female	0/10	0/10	0/10	1/10	10/10
Single cell hepathocytic necrosis	Male	0/10	0/10	0/10	2/10	5/10
	Female	0/10	0/10	0/10	3/10	7/10
Periacinar hepathocytic eosinophilia	Male	0/10	0/10	0/10	7/10	8/10
	Female	0/10	0/10	0/10	3/10	1/10
Centriacinar hepathocytic eosinophilia	Male	0/10	0/10	0/10	0/10	6/10
	Female	0/10	0/10	0/10	0/10	8/10

Conclusions

Treatment of mice for 13 weeks with cyproconazole at ≥ 300 ppm caused retardation of body weight gain particularly among male mice; liver enlargement in males and females; and histopathological liver changes in males and females. Some histopathological changes were indicative of a disturbance of lipid metabolism in the liver. There was an increased incidence of single cell necrosis, and of hepatocyte eosinophilia. The increased incidence of single-cell necrosis at the two higher dose levels, and the retardation of weight gain among males indicate that both of these levels of treatment are excessive for a long-term study. Both 15 ppm and 5 ppm showed no treatment-related effects. The dose of 15 ppm is therefore the no effect level (NOEL) in this study, corresponding to 2.2 mg/kg bw/day in males, and 3.2 mg/kg bw/day in females. However, it should

be recognised that the spacing between the NOAEL and the LOAEL (300 ppm) was very large. The "real" LOAEL for this study is expected to be somewhere in-between 15ppm and 300ppm. Therefore, due to the inappropriate dosing range and the lack of both clinical and blood chemistry, this study was considered supplementary.

Study 5. 90-day study in the dog (Warren, S., 1986) DAR Vol 3 B.6.3.2.4).

Table 22:   Summary of study findings

Study	Findings
Dog 90-day, feeding, Cyproconazole 95.6% 20, 100, 500 ppm (equiv 0.77/0.7, 4/3.25, 18.8/19.17 mg/kg d males/females)	**500 ppm (8-18.8/19.17 mg/kg bw/day)** Body weight and food consumption: ↓body weight gain (wks 0-13) in males (-33%) and females (-58%). ↓terminal body weight (-11% and -9%) for males and females respectively. ↓food consumption wk 1-6 males (-20) Haematology: -slight reduction in RBC parameters (HGB, HCT and RBC) at the study initiation (week 0); >7% reduction in males at wk 13. ↑platelets in males (40% and 74%, wks 4 and 13, respectively) and females (35% and 44%, wks 4 and 8) Clinical chemistry: . See Table 24 below ↑#glucose, creatinine, ↓bilirubin, cholesterol, triglycerides, total protein, albumen, -altered electrolytes ↑ALAT, AP, GGT, CPK (males), GLDH (+1000%/+250%, in males/females). Organ weight: ↑abs/rel liver weight (**) in males in females ↓rel spleen (+50%) in males and (+22%) in females (**) ↓abs/rel ovary weight Pathology: ↑ accentuated hepatic lobular pattern in males and females Histopathology: ↑mild/moderate hepatomegaly + single cell degeneration in females ↓follicular activity (ovary) -inactive endometrium See Table 24) **≥100 ppm (43.8/70.2 mg/kg bw day)** Body weight and food consumption: ↓body weight gain wks 0-13 in males (-43%) and (-22%) in females. ↓terminal body weight (-9.5%) for males. ↓food consumption in males. Organ weight: ↑abs/rel liver weight in males in females ↑abs/rel ovary weight Histopathology: ↑mild hepatomegaly (see below). **20 ppm (0.77/0.7 mg/kg bw/day)** Organ weight ↑abs/rel liver weight (males +14%/+13%; females +17%/+18%)

values fluctuated widely, considered not-toxicologically relevant. See Table 23 below).

Clinical Chemistry

Table 23: 90-day dog Altered clinical chemistry parameters

Parameter		control		20 ppm		100 ppm		500 ppm	
		male	female	male	females	males	female	males	female
creatinine	0	80.6	79.7	66.8	66.9	73.9	75.9	81.5	84.6
	4	68.6	68.6	67.3	70.0	82.4**	83.6*	82.4**	81.9*
	8	70.5	62.6	64.8	67.2	72.8	71.8*	69.3	69.9
	13	75.9	72.9	75.3	73.6	84.0	81.7	79.3	83.8
bilirubin	0	1.32	1.26	1.34	1.73	1.05	1.70	1.25	1.57
	4	0.82	1.04	1.02	1.25	0.83	1.13	0.58	0.52
	8	1.15	1.16	1.23	1.45	1.05	1.44	0.79	0.67
	13	1.71	1.63	1.64	2.07	1.64	1.98	1.25	1.30
cholesterol (Roche kit)	0	2.82	2.57	2.79	3.13	3.04	2.44	2.97	2.82
	4	2.74	2.62	3.76	4.06**	3.63	3.20	0.77**	0.75**
	8	3.87	3.07	4.06	5.21**	3.98	3.61	1.64**	1.90
	13	3.66	3.39	3.87	5.16**	3.78	3.64	1.72**	2.25*
cholesterol (Boehringer kit)	8	4.20	3.44	4.31	5.71*	4.51	4.19	2.04**	2.56
	13	3.97	3.83	4.36	6.15**	4.47	4.35	2.08**	2.79
HDL-cholesterol (Boehringer kit)	8	4.57	3.65	4.61	6.34**	4.81	4.47	2.28**	2.82
	13	4.29	4.64	5.42	5.22	4.56	3.25	2.91	2.57
triglycerides	0	0.453	0.378	0.583	0.568	0.413	0.475	0.465	0.513*
	4	0.500	0.485	0.565	0.610	0.418	0.468	0.258*	0.243**
	8	0.443	0.478	0.495	0.563	0.420	0.440	0.313	0.335
	13	0.373	0.400	0.443	0.565	0.383	0.505	0.300	0.365
total protein	0	59.2	59.6	58.3	57.9	57.9	57.6	58.4	57.8
	4	58.7	61.1	61.6	62.4	59.9	60.4	55.3	54.9*
	8	53.1	55.2	55.4	56.1	54.1	54.6	49.9	49.5*
	13	60.9	62.2	62.5	63.8	63.9	64.2	57.4	59.3
albumin	0	29.7	29.5	28.4	29.3	28.4	28.6	29.4	28.3
	4	30.1	30.6	29.8	31.7	28.1	30.6	24.6**	24.3**
	8	30.5	31.9	30.4	33.1	28.6*	31.1	24.1*	24.4**
	13	30.8	30.7	29.8	31.5	28.8	30.0	23.5**	24.0*
calcium	0	2.24	2.18	2.24	2.23	2.23	2.23	2.14	2.14
	4	2.65	2.58	2.60	2.63	2.67	2.54	2.36*	2.41
	8	2.64	2.61	2.54	2.59	2.56	2.57	2.38	2.42
	13	2.53	2.51	2.47	2.54	2.47	2.51	2.32*	2.30*
phosphate	0	2.40	2.23	2.50	2.30	2.35	2.14	2.28	2.07
	4	3.93	3.73	4.19	4.18	3.96	3.74	3.17*	2.94*
	8	2.79	2.65	2.96	2.97	2.81	2.65	2.25*	2.09*
	13	1.74	1.72	1.87	1.65	1.68	1.61	2.02	1.50
ALAT	0	36.0	28.3	31.1	26.5	28.3	24.5	29.1	28.3
	4	35.8	26.5	28.7	24.4	26.2	23.7	35.5	30.7
	8	26.8	27.2	27.4	24.5	25.2	22.4	38.7**	25.6
	13	32.7	23.4	29.0	22.9	25.5	20.9	41.8	28.1
AlP	0	180.5	210.0	206.3	166.8	188.8	242.0	201.3	209.8
	4	142.3	181.3	188.3*	138.5	151.3	230.0	394.8*	349.0**
	8	121.5	149.8	167.0	132.1	132.8	188.8	447.3*	466.5*
	13	88.6	118.9	137.7	99.2	119.3	156.1	459.3**	456.0**
γ-GT	0	0.98	1.15	1.32	0.95	1.22	1.27	1.76	1.72
	13	2.76	2.41	2.24	2.43	2.79	4.09*	4.33*	4.13*
CPK	4	85.5	97.3	119.3	95.8	100.0	109.0	165.3*	126.5
	8	81.2	96.8	109.3	83.2	106.7	98.8	146.9	112.6

Parameter		control		20 ppm		100 ppm		500 ppm	
		male	female	male	females	males	female	males	female
	13	49.0	59.7	69.0	50.5	63.5	57.8	86.4	61.3
GLDH	0	4.46	3.57	5.18	3.66	3.65	3.36	3.90	4.53
	13	0.36	0.98	1.50	2.07	1.28	0.57	4.46**	3.56*

*) P<0.05   **) P<0.01

Additional blood samples were collected in the last week of treatment in the morning before feeding the animals (a.m.) and about four hours later again (p.m.) in order to investigate possible changes in blood cortisol and testosterone levels shortly after the ingestion of test substance. Whereas there was an obvious increase *vs.* control in the high dose group both in the morning and at noontime, cortisol levels in the low dose group, which were about twice the control values in the morning, did not differ significantly from controls at noon. The intermediate dose affected plasma cortisol only minimally. To summarise, plasma cortisol levels were found to be higher in treated animals of all groups than in the controls. However, there was no obvious dose relationship.

Pathology

Table 24:   90-day dog Summary organ weights

Organ		Males				Females			
		control	20 ppm	100 ppm	500 ppm	control	20 ppm	100 ppm	500 ppm
carcass	(kg)	11.73	12.99	11.75	10.23	8.92	10.72*	9.62	7.98
Liver	absolute (g)	308	316	387	441**	261	293	265	248**
	rel. to bw (%)	2.6	2.5	3.3	4.3**	2.9	2.8	2.8	4.4**
Brain	absolute (g)	81.3	82.2	79.8	76.0	76.3	80.7	75.5	83.2
	rel. to bw (%)	0.7	0.6	0.7	0.7	0.9	0.8	0.8	1.0**
Ovaries	absolute (g)	-	-	-	-	1.274	2.320	2.387	0.698
	rel. to bw (%)	-	-	-	-	0.014	0.021	0.025	0.009

Conclusion:
Minor changes in clinical chemistry parameters which fluctuated considerably were seen at 100 ppm. Also seen were changes in absolute and relative liver weight, which are considered treatment related adaptive responses. At 500 ppm there were clear changes in clinical chemistry parameters (bilirubin, cholesterol, albumin, etc.) as well as a significant increase in the number of platelets. These changes in clinical chemistry parameters can possibly be associated with interference with the adreno-corticosteroid synthesis pathway as similarly observed in a previous three month feeding study in the rat (Gerspach, April 1999; B.6.3.2.2). However histological examination revealed no changes in the adrenal gland. A number of these changes have also been associated with toxic effects on the liver and due to their slight recovery over the study progression indicate a reversible response. The increased liver weight and hepatomegaly with some hepatocyte degeneration seen at 500 ppm, supported by alterations in liver clinical chemistry endpoints, indicates a toxic effect consistent with initiation of liver damage due to exposure to cyproconazole.

 4.7.1.2 Repeated dose toxicity: inhalation

Study 1. 16-day rat (Bernstein D, Luetkemeier H., Vogel, O, et al., 1987) DAR Vol 3 B.6.3.3.2

Table 25: **Summary of study findings**

Study	Findings
Rat 16-day, nose only inhalation, Cyproconazole 96.2% 0.01, 0.1, 1.00 mg/L	**1.0 mg/l**: Mortality/clinical signs: 3/5 females died; all remaining animals sacrificed within 6 days. All animals -hunched posture, laboured respiration, ruffled fur and weakness. Ventral recumbence in females only. Pathology: -haemorrhage in lungs (1 male/4 females) -perivascular cuffing/mineral deposit (3 males), -thrombosis and emphysema (2 females) Histopathology: Table 28 -slight squamous metaplasia of nasal epithelium 2/5 females) ↑mild/moderate hepatocellular hypertrophy (4 males/2 female) ↑fatty change hepatocytes (5 males/4 females) ↑vacuolation of macrophates in splenic pulp (2 males/2 females) ↑depletion of lymphocytes in spleen (4 females) **0.1 mg/l**: Body weight and food consumption: ↓body weight gain (wks 0-13) in females ↓terminal body weight (-12%) females Slight ↑food consumption and body weight/gain in males Haematology: - ↑WBC after 3 wks recovery (males/females) Clinical chemistry: . See Table 27 ↓bilirubin males only. ↑ASAT/ALAT/LDH females only. All reversible Organ weight: ↑abs/rel liver weight (**) in males ↑rel liver weight in females (**) ↑abs/rel kidney weight (**) in males Histopathology: Table 28 ↑mild/moderate hepatocellular hypertrophy (2 males/1 female) **0.01 mg/l:** Some clinical signs

*P<0.05; ** P<0.0

Table 26:   Relevant Clinical chemistry parameters

Parameter		Control	0.01 mg/L	0.1 mg/L	Control	0.01 mg/L	0.1 mg/L
		Males			Females		
bilirubin	week 3	9.2	7.3*	7.6*	8.0	8.5	9.1
	recovery			10.7			12.0
LDH	week 3	204	181	157	158	190	373
	recovery			160			75
ALP	week 3	173	170	146	75.0	62.8	64.6
	recovery			114			62.6
ASAT	week 3	87.6	80.8	81.5	76.5	72.1	86.9
	recovery			67.7			58.4
ALAT	week 3	66.6	64.9	65.6	43.0	42.0	61.3
	recovery			44.6			38.1

*) $P < 0.05$

Table 27:   Histopathological findings in decedent animals

Parameter	Control	1 mg/L	Control	1 mg/L
	Males		Females	
Nasal epithelium				
Squamous metaplasia				2/5
Liver				
Hepatocellular hypertrophy		4/5		2/5
Fatty change	1/5	5/5	1/5	4/5
Spleen				
Vacuolation of macrophages		2/5		2/5
Depletion of lymphocytes				4/5

Table 28:   Histopathological findings in surviving animals

Parameter	Control	0.01 mg/L	0.1 mg/L	Control	0.01 mg/L	0.1 mg/L
	Males			Females		
Liver						
Hepatocellular/ hypertrophy week 3		1/3	2/3			1/3
Spleen						
Haemosiderosis		0/3	0/3		0/3	3/3
recovery						1/3

Conclusion:

The highest aerosol concentration of 1 mg/L caused substantial mortality in male and female rats. The liver, the respiratory tract and the spleen were the target organs at this dose level. At lower concentrations the liver was the main target organ, with changes in liver weights accompanied by slight changes in liver enzymes and hepatocellular hypertrophy and also fatty change. There were changes in the weight of the kidney not associated with alterations in clinical chemistry and only slight mineralisation observed histopathologically. Spleen weight was not recorded, however

histopathological examination revealed effects at the high dose and haemosiderosis in the females at the mid-dose. Haemosidersosis has previously been noted in other sub-chronic studies with cyproconazole and is considered treatment related. Clinical signs observed at the lowest concentration were reversible, this nominal concentration of 0.01 mg/L corresponding to an achieved concentration of 0.017 mg/L is considered to represent the NOAEL of the study. On a body weight basis this concentration corresponds to 4.9 mg/kg bw/day (6 hours exposure per day; 0.8 L air/kg/min).

4.7.1.3 Repeated dose toxicity: dermal

Study 1: 28-day dermal study in the rat (Sommer, 2000) DAR Volume 3 B.6.3.3.1

Table 29:   Summary of study findings

Study	Findings
Rat 28-day, dermal, Cyproconazole 95.5% 0, 10, 100 or 1000mg/kg bw 6 hours/day, 5days/week for 3 weeks and then daily to termination.	**1000 mg/kg bw** Mortality/clinical signs None Body weight and food consumption: Not affected Haematology: ↑WBC after 3 wks recovery (3 males) ↑prothrombin time (**) ↑haemoglobin distribution width (**) ↑relative monocytes (**) ↓basophils (ns) Clinical chemistry: ↓globulin..↓albumin/globulin ↑total protein (*in females; ns in males) ↓bilirubin (**males; ns in females). ↑cholesterol (ns males; **females) ↑ASAT/ALAT/LDH males only. Organ weight: ↑abs liver weight (**) in males (20%)/females (26%) ↑rel liver weight((**) in males (25%)/females (28%) Histopathology: ↑acanthosis application site (males/females) ↑Centrilobular hepatocellular hypertrophy ↑splenic haemosiderosis ↑thyroid follicular hypertrophy **100 mg/kg bw** Haematology: ↓basophils (ns) Clinical chemistry: . ↓globulin.(females). ↑cholesterol (females ns) Histopathology: Table 29 ↑acanthosis application site (males/females) ↑Centrilobular hepatocellular hypertrophy (males) ↑splenic haemosiderosis (females) ↑thyroid follicular hypertrophy (males/females) **10 mg/kg bw** No effects

Table 30:   Treatment-related microscopic findings

		Males				Females			
Dose level (mg/kg)		**0**	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
No. of tissues examined		10	10	10	10	10	10	10	10
skin application site	incidence	4	5	9	10	4	3	4	5
acanthosis	*average grade*	*1.3*	*1.4*	*1.3*	*1.6*	*1.0*	*1.0*	*1.3*	*1.4*
liver	incidence	0	0	7	10	0	0	0	5
centrilobular hepatocellular hypertrophy	*average grade*	-	-	*1.0*	*1.8*	-	-	-	*1.0*
spleen	incidence	0	0	0	9	2	1	5	10
haemosiderosis	*average grade*	-	-	-	*1.0*	*1.0*	*1.0*	*1.0*	*1.3*
thyroid gland	incidence	5	5	8	9	5	5	8	9
follicular hypertrophy	*average grade*	*1.6*	*1.4*	*1.3*	*1.8*	*1.0*	*1.0*	*1.1*	*1.7*

Conclusion:

Dermal application of cyproconazole technical to rats at dose levels of 0, 10, 100 and 1000 mg/kg over a 28-day period was well tolerated at all dose levels tested. There were no signs of overt toxicity. Haematological changes including increased prothrombin time, increased monocytes and elevated haemoglobin concentration distribution width (HDW) were observed at 1000 mg/kg. Dose levels of 100 mg/kg and 1000 mg/kg altered blood chemistry parameters in particular in females including increased protein, cholesterol and globulin. The mean liver weights were increased at 1000 mg/kg in both sexes. Centrilobular hepatocellular hypertrophy was observed 100 mg/kg males and 1000 mg/kg males and females. Hypertrophy of thyroid follicular epithelium was seen in animals of both sexes at 100 and 1000 mg/kg. Splenic haemosiderosis was minimal in males and minimal to slight in females and there were no corresponding haematological parameters. Acanthosis is considered a local effect at the application site.

The NOAEL level for systemic effects was set at 10 mg/kg body weight. Effects seen at 100 mg/kg bw were reflective of adaptive response in both the liver and thyroid and not considered adverse *per se.* The LOAEL is seen at 1000 mg/kg bw based on the range of effects observed (haematology, clinical chemistry and histopathology) including significantly enlarged liver in male and females.

4.7.1.4 Repeated dose toxicity: other routes

None

4.7.1.5 Human information

None available

4.7.1.6 Other relevant information

None available

4.7.1.7 Summary and discussion of repeated dose toxicity

The toxicological properties of cyproconazole upon short-term treatment were investigated in rat, mouse and dog. In all species investigated, liver was the main target organ for cyproconazole. Evidence for a disturbance of lipid metabolism was found in all species. A minimal anaemia was observed at the highest dose level investigated in dogs and evidence for a disturbance of the haematopoietic system was reproducible in rats at or above the MTD level; however, without a clear manifestation of anaemia in this species.

The LOAEL was found in rats at 1000 ppm (96.18/127.59 mg/kg bw/day) after 28 days of treatment and at 350 ppm (24.7/29.6 mg/kg bw/day) after 90 days of treatment, based on body weight effects and functional and histopathological changes in the liver. The dose of 300 ppm (43.8/70.2 mg/kg bw/day) was an LOAEL in the 90-day mouse study, based on body weight effects and histopathological liver changes. The liver was the target in the dog 90-day study with increased liver weight and hepatomegaly with some hepatocyte degeneration seen at 500 ppm (18/19 mg/kg), supported by alterations in liver clinical chemistry endpoints, indicative of a toxic effect in the liver.

The target organ at non-lethal concentrations in the 16-day inhalation study in rats was the liver, indicated by slight, reversible changes in liver enzymes and hepatocellular hypertrophy. The highest aerosol concentration of 1 mg/L caused substantial mortality in male and female rats. The liver, the respiratory tract and the spleen were the target organs at this dose. At lower concentrations the liver is indicated as target organ, since changes in liver weights were accompanied by slight changes in liver enzymes and hepatocellular hypertrophy. The weak effects observed at the lowest concentration were considered completely reversible (NOAEL = nominal concentration of 0.01 mg/L corresponding to an achieved concentration of 0.017 mg/L). On a body weight basis this concentration corresponds to 4.9 mg/kg bw/day (6 hours exposure per day; 0.8L air/kg/min). The NOAEL of 0.017 mg/L corresponds on a body weight basis to 4.9 mg/kg bw/day.

Dermal application of cyproconazole technical to rats at dose levels up to 1000 mg/kg over a 28-day period was well tolerated without any signs of overt toxicity. Liver weight with hepatocellular hypertrophy was significantly increased at 1000 mg/kg bw. Clinical chemistry indicated some alteration in liver function. At 100 mg/kg there were changes in haematological parameters and clinical chemistry findings (indicating liver) including increased cholesterol, protein and globulin.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

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

Classification in STOT Cat 2 is required when:

....on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

The summary data from the sub-chronic testing of cyproconazole in rats, mice and dogs clearly identify the liver as the main target organ with evidence of adaptive change but also with evidence of hepatocyte toxicity (See Table 15).

Adverse effect levels were generally low, with evidence of significant hepatotoxicity seen at dose levels below the cut-off levels in the rat (28-day (oral and dermal), 90-day oral, and inhalation (16-day), mouse (90-day) and dog (90-day/1 year). Increases in relative liver weight were seen with histopathological change such as hepatocellular hypertrophy, vacuolation, fatty change, and single cell necrosis. These effects were generally accompanied by evidence of functional impairment such as altered clinical chemistry and marker enzymes. The significant adverse effects in the three species are consistent with the criteria for STOT RE 2 H373 (May cause damage to organs (liver)) with the exception of the 28 day rat study where the findings were more severe (at 1000 ppm: 96.18/127.9 mg/kg). The findings of the inhalation and dermal route studies in the rat also support this conclusion.

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

The significant adverse effects in the three species are consistent with the criteria for STOT RE 2; H373 (May cause damage to organs (liver)) in the basis of evidence of significant liver toxicity below the cut-off values in three species tested.

CLP: STOT RE 2; H373

4.9 Germ cell mutagenicity (Mutagenicity)

Table 31: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Reference
In vitro		
Procaryote gene mutation		
In vitro microbiological assay; *S. typhimurium* TA 98, TA 100, TA 1538, TA 1535 and TA 1537 -95.6%: 1.0-5000 μg/plate	Negative	Hoorn, A. (1986) DAR Vol 3; B.6.4.1.1)
mitotic non-disjunction assay; *Saccharomyces cerevisiae* -10-550 μg/ml (Purity not specified)	Negative +/- S9	Hoorn, A. (1985) DAR Vol 3 (B.6.4.1.7)
Ames test; *S. typhimurium and E Coli* reverse mutation assay; TA 1535, TA 1537, TA 98, and TA 100 and WP2 uvrA pKM 101, and WP2 pKM 101. -3, 10, 33, 100, 333, 1000, 2500, and 5000 μg/plate (purity 96.6%)	Negative +/- S9	Sokolowski A (2009) DAR Addendum 2009 (B.6.4.1)
Mammalian gene mutation		
HGPRT gene mutation assay; Chinese Hamster cell line V79 -20-200μg/ml (purity 94.4%)	Negative +/- S9	Miltenburger, H. (1985b)
Chromosomal aberrations		
In vitro mammalian cytogenetic test; Chinese hamster ovary (CHO cells) -100-200μg/ml (95.6%)	Weakly clastogenic +/- S9 Re-evaluation: negative +/- S9	Enninga, I.C. (1988) McEnaney, S. (1992) – re-evaluation (B.6.4.1.2/3),
In vitro mammalian cytogenetic test; Chinese hamster ovary (CHO cells) -45-150 μg/ml (95.6%)	Negative +/- S9	Murli, H. (1990) (B.6.4.1.4)

Method	Results	Reference
In vitro		
In vitro mammalian cytogenetic test; Chinese hamster ovary (CHO cells) -100-800 μg/ml (100%)	Negative +/- S9	Saigo, K. (1995) (B.6.4.1.5)
DNA repair *in vitro*/cell transformation assay-mammalian		
In vitro mammalian cell DNA repair test; Rat primary hepatocytes -0.15-15μg/ml (96.2%)	Negative +/- S9	Curren, R. (1988) (B.6.4.1.8)
In vitro cell transformation assay Syrian hamster embryo (SHE) cells -20-200 μg/ml (94.4%)	Negative +/- S9	Miltenburger, H.G. (1985a) (B.6.4.1.6)
In vivo		
Micronucleus assay		
in vivo mouse micronucleus assay; Swiss random mice (5m/ 5f/group) -0, 16.7, 55.7, 167 mg/kg (purity not specified)	Negative +/- S9	Taalman, R. (1985) (B.6.4.2.1)
In *vivo* mammalian bone marrow; cytogenetic test ICO:CDI (CRL) mice (5m/5f/group) -0, 50, 100, 200 mg/kg (purity 95.5%)	negative +/- S9	Ogorek, B. (1999) (B.6.4.2.2)
Germ cell assays		
Dominant lethal study; SD rats (20 m/group) -0, 20, 40, 80 mg/kg (95.6%)	Negative +/- S9	Putman, D.L. (1991) (B.6.4.3.1))

4.9.1   Non-human information

4.9.1.1 *In vitro* data

Cyproconazole technical did not induce gene mutations in bacterial or in mammalian cells *in vitro*. At first the *in vitro* chromosome aberration test in CHO cells revealed some evidence for the clastogenic potential of cyproconazole. However, this finding could not be confirmed when the slides were re-evaluated by a second, independent investigator. Furthermore, two additional *in vitro* chromosome aberration tests - one on CHO, another one on CHL cells – revealed clear negative

results. Cyproconazole did not show evidence for an aneugenic activity in yeast, nor did it induce unscheduled DNA synthesis in rat hepatocytes *in vitro* and it caused no transformation of SHE cells *in vitro*.

An additional Ames test was submitted (Sokolowski, 2009) during the 91/414 re-review to address concerns raised about impurities which are present in the current technical material but which were not tested in the original toxicological data set. This test was negative.

4.9.1.2 *In vivo* data

Cyproconazole was investigated in three *in vivo* studies in mice. Both the bone marrow micronucleus test and a chromosome aberration assay were negative. In addition, cyproconazole was negative in a dominant lethal assay in male germinal cells.

4.9.2   Human information

None available

4.9.3   Other relevant information

None relevant

4.9.4   Summary and discussion of mutagenicity

Cyproconazole was negative in all systems tested *in vitro* and *in vivo*.

4.9.5   Comparison with criteria

Not relevant

4.9.6   Conclusions on classification and labelling

No classification.

4.10   Carcinogenicity

Table 32:   Summary table of relevant carcinogenicity studies

Method	Results	Target organ/ principal effect at LOAEL	Reference
2-year Combined chronic toxicity/oncogenicity Rat/ KFM Wistar SAN 619 F (Purity 95.6% ± 1%) 0, 20, 50, 350 ppm	**NOAEL= 50 ppm** (♂:2.22 mg/kg/day; ♀: 2.73 mg/kg/day) **LOAEL = 350 ppm** (♂: 15.59 mg/kg/day; ♀: 21.76 mg/kg/day)	**350 ppm** <u>♂'s:</u> Reduced bodyweight gain; <u>Liver:</u> Increased incidence and severity of fatty change, decreased bilirubin, increased plasma γ-GT, ALAT and ASAT <u>♀'s:</u> Reduced bodyweight gain; <u>Liver:</u> Increased relative liver weight; increased incidence of hepatocellular hypertrophy;	Warren , S. F. P. *et al.* (1988); Report no. 357-R; AGRO DOK CBK I.6858/87 DAR Vol 3 B.6.5.1

Method	Results	Target organ/ principal effect at LOAEL	Reference
		decreased bilirubin, increased plasma γ-GT and cholesterol levels Heart: myocardial necrosis/fibrosis and an increase in relative heart weight **50 ppm** No effects	
18-month Oncogenicity Mouse/CD-1 SAN 619 F (95% ± 1%) 0, 5, 15, 100, 200 ppm	**NOAEL= 15 ppm** (♂: 1.84 mg/kg/day; ♀: 2.56 mg/kg/day) **LOAEL=100 ppm** (♂: 13.17mg/kg/day; ♀: 17.65mg/kg/day)	**200 ppm** ♂'s: Reduced bodyweight gain; Liver: Increased incidence of adenomas and carcinomas, increased relative liver weight, increased incidence of accentuated lobular pattern, increased incidence of focal hepatocytic inflammation, single cell necrosis and diffuse hypertrophy Testes: increased incidence of germinal epithelium deficit, and aspermia of the epididymis ♀'s: Reduced bodyweight gain; Liver: Increased relative and absolute liver weight; increased incidence of accentuated lobular pattern, increased incidence of single cell hepatocytic necrosis, centriacinar and periacinar vacuolation. 100 ppm Reduced body weight gain. Increased rel. liver weight (males and females) increased incidence of accentuated lobular pattern, of focal hepatocytic inflammation (males), single cell necrosis (both) and diffuse hypertrophy, centriacinar and periacinar vacuolation (females).	Warren , S. F. P. *et al.* 1989; Report no. 388-M; AGRO DOK CBK I.7171/89 DAR Vol 3 B.6.5.2

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Study 1: Oral carcinogenicity study in the rat

Administration of cyproconazole at target dietary doses of 0, 20, 50 or 350 ppm to KFM Wistar rats for a period of 2 years did not result in any treatment-related mortalities or clinical signs of toxicity. Body weight gain in high dose animals however, was clearly impaired by treatment from week 2 onwards, the effect being more pronounced in females (final bodyweights of males and females from the high dose group were 5.1% and 13.3% lower than controls, respectively). A reduction in food consumption was not observed in any test group; in fact consumption actually increased in

high dose females during the first 13 weeks by 7% compared to the control group (though this apparent increase may partially be due to food scattering).

The liver appears to be a target organ for cyproconazole in rats, with hepatic effects occurring in both sexes at the high dose level (350 ppm). The principle effects occurring in males were an increase in the incidence and severity of fatty change, which occurred in all three sacrifice groups (weeks 52, 78 and 118). These fatty changes were not associated with degenerative hepatic lesions. Associated clinical chemistry alterations in high dose males included decreased bilirubin levels, increased gamma-GT activity and increased ALAT and ASAT. In addition, relative liver weights of high dose males were slightly increased (up to 10% increase, relative to controls at week 78) but not statistically significant. In females, hepatic effects included an increased incidence of hepatocellular hypertrophy which was found in 44% (4/9 animals) of high dose females of the interim sacrifice (week 78) and a statistically significant increase in relative liver weights (all sacrifice groups). The absolute liver weights of high dose females were also increased relative to controls at week 78 and at terminal sacrifice but the increase was not statistically significant. These hepatic changes in high dose females were accompanied by decreased bilirubin levels, increased gamma-GT activity and increased cholesterol levels.

There was an increase in the incidence (and a slight increase in severity) of necrosis/fibrosis of the heart in females from all treatment groups at week 78 and at study termination. At study termination, the pattern of increase appeared to be dose related. There was also an increase in relative heart weight in high dose females at both of these sacrifice time-points, though absolute heart weights did not appear to be affected. In addition, a statistical increase in total proteins and total globulins was observed in high dose females at sacrifice week 78 and at study termination and total proteins also were significantly increased at 50ppm in females at study termination. The toxicological significance of these findings is uncertain however. CPK levels (usually a marker for cardiotoxicity) were actually reduced in high dose females at week 78, though not to a statistically significant extent (though standard deviations in CPK levels in general were very large). LDH levels were not measured. Myocardial necrosis/fibrosis is a common finding among aged rats. The apparent cardiac effects at the highest dose level (myocardial necrosis/fibrosis and an increase in relative heart weight) are unlikely to be treatment-related.

There was no evidence of an effect of cyproconazole on the endocrine system. Corticosterone levels measured at week 52 were unaffected and there was no disturbance of the tumour profile in endocrine tissues that might have resulted from a disturbed endocrine status.

There was also no evidence of treatment-related tumourigenesis. It is therefore concluded that, under the conditions employed, cyproconazole is not carcinogenic in Han Wistar rats.

Based on reduced bodyweight gain, liver changes at 350 ppm, the NOAEL in this study is considered to be 50 ppm corresponding to 2.22 and 2.73 mg/kg bw/day in males and females, respectively.

Table 32-1: Notable changes in terminal bodyweight, absolute organ weights and relative organ weights (% of bw) of rats administered cyproconazole.

Parameter	Week	Dose level (ppm)							
		Males				Females			
		0	20	50	350	0	20	50	350
Terminal Bodyweight (g)	Week 52	577	580	563	533	333	299	315	266*
	Week 78	593	610	635	587	355	354	370	301*
	Week 118/121	533	546	580	512	388	371	389	326*
Absolute Liver Weight (g)	Week 52	14.44	14.54	13.83	13.90	8.71	8.06	8.86	8.13
	Week 78	14.94	15.68	16.23	16.24	8.58	8.81	9.86	9.55
	Week 118/121	17.0	16.3	17.6	18.2	13.3	13.0	13.1	13.9
Relative Liver Weight (%)	Week 52	2.51	2.51	2.46	2.61	2.62	2.70	2.82	3.06**
	Week 78	2.52	2.58	2.56	2.77	2.46	2.50	2.69	3.19**
	Week 118/121	3.26	3.02	3.06	3.55	3.43	3.58	3.44	4.31**
Absolute Kidney Weight (g)	Week 52	3.017	3.057	2.882	2.774	1.994	1.841	1.877	1.729
	Week 78	3.148	3.581	3.512	3.178	2.037	2.095	2.313	2.066
	Week 118/121	4.46	4.29	4.03	3.93	2.62	2.83	2.78	2.70
Relative Kidney Weight (%)	Week 52	0.53	0.53	0.52	0.52	0.60	0.62	0.60	0.65
	Week 78	0.53	0.59	0.56	0.54	0.58	0.60	0.63	0.69*
	Week 118/121	0.89	0.81	0.70	0.77	0.68	0.80	0.73	0.85**
Absolute Heart Weight (g)	Week 52	1.343	1.421	1.297	1.274	0.909	0.899	0.918	0.815
	Week 78	1.403	1.452	1.456	1.351	0.944	0.964	1.030	0.968
	Week 118/121	1.62	1.66	1.77	1.66	1.22	1.22	1.26	1.35
Relative Heart Weight (%)	Week 52	0.234	0.245	0.232	0.239	0.277	0.301	0.293	0.307
	Week 78	0.237	0.240	0.230	0.231	0.270	0.274	0.282	0.324**
	Week 118/121	0.31	0.31	0.31	0.33	0.32	0.33	0.33	0.43**
Absolute Brain Weight (g)	Week 52	2.157	2.202	2.146	2.151	1.957	1.976	1.955	1.888
	Week 78	2.134	2.131	2.131	2.094	1.958	1.983	2.008	1.991
	Week 118/121	2.25	2.26	2.22	2.21	2.03	2.02	2.01	2.05
Relative Brain Weight (%)	Week 52	0.377	0.381	0.384	0.405	0.603	0.669	0.625	0.714**
	Week 78	0.363	0.355	0.338	0.359	0.566	0.565	0.554	0.668*
	Week 118/121	0.43	0.42	0.39	0.44	0.54	0.56	0.53	0.65*

*P<0.05   **P<0.01*

Non-neoplastic findings:

Table 32-2:   Incidence (no. and %) of selected histopathological (non-neoplastic) changes in rats administered cyproconazole.

Organ	Sacrifice	Dose level (ppm)							
		Males				Females			
		0	20	50	350	0	20	50	350
– Liver									
Fatty change	Week 52	4/10 (40%)	5/10 (50%)	7/10 (70%)	10/10** (100%)	1/10 (10%)	0/10 (0%)	0/10 (0%)	1/10 (10%)
	Week 78	3/10 (30%)	1/10 (10%)	4/10 (40%)	10/10 (100%)**	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/9 (0%)
	Week 118/121	23/50 (46%)	19/49 (39%)	29/50 (58%)	38/50** (76%)	23/50 (46%)	15/50 (30%)	15/50 (30%)	10/50 (20%)
Hepatocellular hypertrophy	Week 52	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
	Week 78	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	4/9** (44%)
	Week 118/121	0/50 (0%)	0/49 (0%)	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Vacuolated focus	Week 52	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
	Week 78	3/10 (30%)	1/10 (10%)	4/10 (40%)	1/10 (10%)	1/10 (10%)	0/10 (0%)	2/10 (20%)	1/9 (11%)
	Week 118/121	14/50 (28%)	18/49 (37%)	19/50 (38%)	16/50 (32%)	2/50 (4%)	9/50 (18%)	9/50 (18%)	9/50 (18%)
Mixed focus	Week 52	0/10 (0%)	0/10 (0%)	0/10 (0%)	2/10 (20%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
	Week 78	2/10 (20%)	2/10 (20%)	2/10 (20%)	1/10 (10%)	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/9 (0%)
	Week 118/121	11/50 (22%)	8/49 (16%)	9/50 (18%)	10/50 (20%)	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Liver cell pigmentation	Week 52	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	0/10 (0%)

Organ	Sacrifice	Dose level (ppm)							
		Males				Females			
	Week 78	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	6/10 (60%)	5/10 (50%)	8/10 (80%)	8/9 (89%)
	Week 118/121	0/50 (0%)	0/49 (0%)	0/50 (0%)	2/50 (4%)	3/50 (6%)	6/50 (12%)	9/50 (18%)	10/50 (20%)
Sinusoidal cell pigmentation	Week 52	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)
	Week 78	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/9 (11%)
	Week 118/121	1/50 (2%)	1/49 (2%)	4/50 (8%)	5/50 (10%)	5/50 (10%)	7/50 (14%)	6/50 (12%)	13/50 (26%)

*P<0.05   **P<0.0

Study 2: Dietary carcinogenicity and chronic toxicity study in mice.

Chronic toxicity and carcinogenicity were investigated in the mouse in a 88 week feeding study. The test substance was administered as a dietary admixture to four groups of CD-1 mice (50 males and 50 females per group) at target doses of 5, 15, 100 or 200 ppm over a period of 81 weeks (males) or 88 weeks (females). Two duplicate groups of 50 animals/sex/group received untreated diet as a control. In addition two satellite groups (10 animals/sex/group) receiving 0 or 200 ppm were used for interim sacrifice at week 13.

Table 33-1:   Study design

Group	Group No.	Dose (ppm)	Number of Males in group	Number of Females in group
Main Control	K	0	50	50
Satellite control	K1	0	10	10
2nd Control group	P	0	50	50
Low dose	A	5	50	50
Intermediate dose 1	B	15	50	50
Intermediate dose 2	C	100	50	50
Main High dose	D	200	50	50
Satellite High dose	D1	200	10	10

Mean and maximum achieved daily dietary intake of test substance in mice administered SAN 619 F for 81 weeks (males) or 88 weeks (females) were as follows;

Table 33-2:   Dietary intake

Dose (ppm)	Group	Mean and highest intake of test substance (mg/kg bw/day)	
		Males	Females

		Mean	Highest	Mean	Highest
5	A	0.69	0.90	1.03	1.15
15	B	1.84	2.68	2.56	3.42
100	C	13.17	17.60	17.65	23.17
200	D	27.85	35.24	36.30	46.64
200	D1	33.06	35.88	43.30	48.22

Chronic toxicity: Mortality rate was not increased by treatment and there were no clinical signs of toxicity. In the main study, the number of decedents (that died spontaneously or were sacrificed in extremis) among mice receiving 100 or 200 ppm cyproconazole appeared less than that among the controls or among mice receiving 5 or 15 ppm (Table 33.3).

Table 33-3:   Mortality data.

Dose (ppm)	Group No.	Male Decedents	Female Decedents
0	K	31/50	33/50
0	P	34/50	27/50
5	A	30/50	26/50
15	B	25/50	30/50
100	C	21/50	14/50
200	D	14/50	23/50

Food consumption was not affected and there were no alterations in haematology parameters. Similar to the rat, body weight development in the mouse was affected by cyproconazole, though in this case, the affect was greater in males. A clear retardation in bodyweight development, relative to controls was observed in both sexes at 100 and 200 ppm, in males from week 13 onwards, in females, after 26 –52 weeks of treatment.

Table 33-4:   Cumulative bodyweight gain (gms and as % of control group 1 (groupK)) at various time points in mice administered SAN 619 F in their diet for 18 months.

Dose (ppm)	Group	Bodyweight gain (g) and % of controls							
		Male				Female			
		Week 0-13	Week 0-26	Week 0-52	Week 0-81	Week 0-13	Week 0-26	Week 0-52	Week 0-88
0	K	8	12	14	16	5	7	10	13
0	P	8 (100%)	12 (100%)	14 (100%)	14 (88%)	5 (100%)	7 (100%)	10 (100%)	11 (85%)
0	K1	9 (113%)	-	-	-	6 (120%)			
5	A	7 (88%)	10 (83%)	12 (86%)	14 (88%)	5 (100%)	7 (100%)	9 (90%)	9 (70%)
15	B	8 (100%)	11 (92%)	14 (100%)	15 (94%)	5 (100%)	8 (114%)	10 (100%)	11 (85%)
100	C	5 (63%)	8 (67%)	10 (71%)	10 (63%)	5 (100%)	6 (86%)	8 (80%)	9 (70%)
200	D	6 (75%)	8 (67%)	11 (79%)	10 (63%)	4 (80%)	6 (86%)	8 (80%)	9 (70%)
200	D1	6 (75%)	-	-	-	5 (100%)	-	-	-

Organ weights:
Absolute and relative liver weights of the satellite groups (200 ppm) terminated after 13 weeks of treatment were significantly increased in males (41% and 53%) and in females (45% and 46%).

At terminal sacrifice relative liver weights of males and females which had received 100 or 200 ppm were again significantly increased; in males at 100 ppm by 22% and at 200 ppm by 42%, and in females by 29% and 59%, respectively. Absolute liver weights were increased by 26% in high dose males and by 24% and 49% in females at 100 and 200ppm, respectively. Since the presence of liver modules might be considered to have an effect on the liver weights, an additional analysis was performed excluding the weights of all organs with a nodule greater than 10 mm diameter. This analysis confirmed increased relative liver weights at 200 ppm (males 37%, females 56%) and at 100 ppm (males 18%, females 27%), which therefore indicates an increase in liver weight that is not a consequence of the nodules.

The apparent significant decrease in absolute kidney weights in both sexes at 100 and 200 ppm relative to controls, were not considered to be treatment related, as relative weight was not affected.

Table 33-5: Terminal bodyweight/absolute organ weights and relative organ weights (% of bw) of mice administered SAN 619 F in their diet for the periods indicated.

Organ	Sacrifice time	Dose (ppm)					
		0(K)	0(P)	5	15	100	200
Males							
Terminal Bodyweight (g)	Week 13	33.8	-	-	-	-	31.3
	Week 81	46.8	44.6	45.2	46.0	41.3**	41.1**
Absolute Liver Weight (g)	Week 13	1.355	-	-	-	-	1.913**
	Week 81	2.554	2.252	2.193	2.426	2.770	3.228**
Absolute Liver Weight (excluding animals with nodules) (g)	Week 81	2.213	2.127	2.164	2.322	2.335	2.696
Relative Liver Weight (%)	Week 13	3.988	-	-	-	-	6.097**
	Week 81	5.488	5.108	4.885	5.332	6.682**	7.789**
Relative Liver Weight (excluding animals with nodules) (g)	Week 81	4.859	4.822	4.824	5.207	5.747*	6.674**
Absolute Kidney Weight (g)	Week 13	0.679	-	-	-	-	0.642
	Week 81	0.967	0.946	0.880	0.895	0.832**	0.830**
Relative Kidney Weight (%)	Week 13	2.042	-	-	-	-	2.056
	Week 81	2.117	2.170	1.958	1.975	2.028	2.027
Females							
Terminal Bodyweight (g)	Week 13	23.88	-	-	-	-	23.19
	Week 81	34.9	33.8	31.5	35.2	33.5	32.4
Absolute Liver Weight (g)	Week 13	1.045	-	-	-	-	1.511**
	Week 81	1.73	1.648	1.536	1.828	2.145**	2.570**
Absolute Liver Weight (excluding animals with nodules) (g)	Week 81	1.73	1.654	1.536	1.685	2.121**	2.493**
Relative Liver Weight (%)	Week 13	4.410	-	-	-	-	6.425**
	Week 81	4.963	4.895	4.878	5.257	6.407**	7.897**
Relative Liver Weight (excluding animals with nodules) (g)	Week 81	4.963	4.932	4.878	4.765	6.322**	7.747**
Absolute Kidney Weight (g)	Week 13	0.396	-	-	-	-	0.378
	Week 81	0.585	0.571	0.533	0.542	0.508**	0.498**
Relative Kidney Weight (%)	Week 13	1.673	-	-	-	-	1.634

Organ	Sacrifice time	Dose (ppm)					
		0(K)	0(P)	5	15	100	200
	Week 81	1.681	1.699	1.700	1.556	1.530	1.541

*P<0.05, **P<0.01

Pathology

Macroscopic change: In the main study there was a significant increase in the incidence of hepatic accentuated lobular pattern and of hepatic masses in the 15, 100 and 200 ppm male and 100 and 200 ppm female groups when compared to each of the two control groups (Table 33-6). The hepatic masses corresponded to the treatment-related increases in hepatic adenomas and carcinomas in both sexes. There was also an increased incidence in 'areas of hepatic change and enlargement' in high dose females, and a slight increase in the incidence of granular liver in the high dose male group. In the satellite group, sacrificed at 13 weeks, one high dose male and one high dose female also had accentuated lobular livers, and two high dose females had pale livers, but none of these findings were statistically significant.

In the gastrointestinal tract of the 100 and 200 ppm male groups there were reduced incidences of those with abnormal contents in all regions examined apart from the stomach, which was unaffected. This treatment-related effect was not seen in females.

Renal findings, which may relate to treatment, were restricted to a reduction in the incidence of granular kidneys in the 200 ppm female group.

There were increased incidences of skin ulceration in all treated groups of both sexes, but only statistically significant in high dose females. Reductions were seen in the incidences of oedema of the sub cutis in male mice of the 100 and 200 ppm dose groups.

There were increased incidences of flaccid testes in the 15 and 200 ppm male groups, though a dose-response relationship was not apparent. These increased incidences corresponded to an increased testicular germinal epithelial deficit in male mice of the 200 ppm dose group only.

Other statistically significant effects included a reduced incidence of excess thoracic fluid in the 100 and 200 ppm male groups and a reduction in the incidence of apparent gall bladder enlargement in the 200 ppm female group.

Table 33-6: Macroscopic changes in mice administered cyproconazole (data is for all animals-died/sacrificed/terminal sacrifice)

Finding		Dose (ppm)					
		0 (K)	0 (P)	5	15	100	200
No of tissues examined		50	50	50	50	50	50
Male							
Liver	- accentuated lobular pattern	3	3	5	10*	13***	23***
	- masses	5	5	5	8	17***	20***
	- areas of hepatic change	2	3	4	3	3	7
	- large appearance	1	3	0	1	2	5
	- granular	0	1	0	0	1	4*
Gastrointestinal tract	- caecum, abnormal content	12	10	9	5	3*	16

	- colon, abnormal content	12	10	10	5	3*	1**
	- rectum, abnormal content	11	11	10	5	3*	1**
	- duodenum, abnormal content	12	11	10	7	3*	4*
	- ileum, abnormal content	12	11	10	8	3*	4*
	- jejunum, abnormal content	12	11	10	8	3*	4*
	- stomach, abnormal content	8	11	12	6	7	5
Skin	- ulceration	6	3	7	7	7	10
	- oedema of subcutis	8	12	12	11	3*	2*
Testes	- flaccid appearance	1	3	4	9**	6	9**
Thorax	- excess fluid	10	12	10	8	4*	3*
Female							
Liver	- accentuated lobular pattern	3	4	4	3	12**	21***
	- masses	1	2	1	3	5	18***
	- areas of hepatic change	3	3	3	0	1	9*
	- large appearance	2	2	1	1	6	10**
	- granular	1	1	0	1	0	3
Gastrointestinal tract	- caecum, abnormal content	8	7	7	6	2	5
	- colon, abnormal content	9	6	5	6	2	6
	- rectum, abnormal content	7	6	4	4	2	5
	- duodenum, abnormal content	7	6	7	5	3	5
	- ileum, abnormal content	7	6	6	5	2	5
	- jejunum, abnormal content	8	5	7	6	2	5
	- stomach, abnormal content	9	7	7	8	5	6
Skin	- ulceration	2	4	6	4	6	9*
	- oedema of subcutis	6	4	3	6	0*	5
Kidney	- granular appearance	21	18	21	17	17	10*
Gall bladder	- enlarged appearance	7	6	2	3	10	0*

*P<0.05, **P<0.01, ***P<0.001   (compared to pooled control K & P)

Histopathology: Non-neoplastic findings – satellite group:
Examination of the satellite group revealed that treatment-related effects following 13 weeks of cyproconazole administration were confined to the liver (Table 33-7). The incidence of periacinar hepatocytic hypertrophy was significantly increased in both sexes at 200 ppm, relative to controls. In addition there was a statistically significant increase in periacinar hepatocytic vacuolation in males and in non-zonal hepatocytic fat vacuolation in females at the high dose level.

Table 33-7: Notable microscopic changes in mice administered cyproconazole in their diet for 13 weeks (satellite group; n = 10)

Finding		Dose (ppm)			
		0	200	0	200
		Male		Female	
No. of tissues examined		10	10	10	9
Liver	- periacinar hepatocytic hypertrophy	4	10*	2	8**
	- periacinar hepatocytic vacuolation	0	5*	2	1
	- non-zonal hepatocytic fat vacuolation (large vacuole)	0	4	0	8***
	- focal inflammation	0	3	2	0

*P<0.05, **P<0.01, ***P<0.001   (compared to control K1)

Non-neoplastic findings – main study:

Consistent with the findings in the satellite group, the major non-neoplastic microscopic changes following long-term administration of cyproconazole, occurred in the liver (Table 33-8). There were a number of adverse changes (focal inflammation, single cell necrosis, hypertrophy, vacuolation) at the two greater dosage levels (100 and 200 ppm) in both sexes, and male mice were more severely affected, in terms of increased incidence. A statistical evaluation of hepatotoxic effects, utilising non-pooled control group incidences, gave essentially very similar results as the pooled results. When using control group 1 alone, there was a loss of significance of focal inflammation in male mice at 100 ppm, of single cell necrosis in the 100 ppm female group, of focal hepatocytic hyperplasia at 200 ppm in females and of centriacinar vacuolation in both sexes. Using control group 2 as the sole reference there were losses of significance as with group 1 as control, plus the loss of significance of periacinar hepatocytic vacuolation at 200 ppm in females. Thus the hepatic non-neoplastic NOEL (15 ppm) was unaffected in male mice and similarly in females.

The following findings also achieved statistical significance at the highest or the two highest dose levels in males: epididymal aspermia (100 and 200 ppm), testicular germinal epithelial deficit (100 and 200 ppm), and testicular amyloid (200 ppm). Other treatment-related, non-neoplastic changes in male mice were an increased incidence (relative to controls) of skin ulceration (200 ppm) and cellulitis (100 and 200 ppm), optic nerve gliosis (200 ppm), and amyloidosis of the ileum (200 ppm) and salivary glands (200 ppm). Long-term cyproconazole administration in males also caused a statistically significant reduction in the incidence of some effects (relative to controls), including pancreatic oedema (200 ppm), interstitial degeneration of the salivary gland (100 and 200 ppm), subcutaneous oedema (200 ppm) and amyloidosis of the spleen (100 and 200 ppm).

Additional treatment-related, non-neoplastic changes in female mice were an increased incidence (relative to controls) of aortic arteritis (200 ppm) and lymphoid hyperplasia in the mesenteric lymph modes (200 ppm). The incidence of a number of findings were reduced compared to controls in females: caecal submucosal oedema (100 and 200 ppm), subcutaneous oedema (100 and 200 ppm), spinal cord compression (200 ppm) and amyloidosis of the kidney interstitium (100 and 200 ppm), the liver (100 and 200 ppm), the spleen (100 and 200 ppm) and the heart (15, 100 and 200 ppm).

Neoplastic findings:

No treatment related neoplasms were found in any of the animals at interim sacrifice at 13 weeks. In the main study, treatment-related neoplastic effects were confined mainly to the liver (Table 34-7) and comprised a significant increase in the incidence of hepatocytic adenoma (in males at 100 and 200 ppm; in females at 200 ppm) and hepatocytic carcinoma (in males at 15 and 100 ppm; in females at 200 ppm). As is evident from Table 34-7, 13% of females that died prior to study termination had hepatic adenomas and 22% had hepatic carcinomas (compared to 0% in controls).

Table 33-8: Microscopic changes in mice (all animals)

Finding		Dose (ppm)					
		0 (K)	0 (P)	5	15	100	200
No of tissues examined		50	50	50	50	50	50
Male							
Liver	- focal inflammation	1	1	1	4	5*	8**
	- single cell necrosis	0	2	2	3	14***	25***
	- diffuse hepatocytic hypertrophy	4	10	4	6	26***	36***
	- centriacinar hepatocytic vacuolation	0	0	0	0	1	3*
	- periacinar hepatocytic hypertrophy	1	0	2	5*	4*	2
	- periacinar hepatocytic vacuolation	0	1	4*	3	1	1
	- amyloid	10	13	4*	12	4*	8
Skin	- ulceration	4	2	5	4	6	9*
	- cellulitis	4	1	5	6	8*	8
	- subcutaneous oedema	7	11	5	12	3	2*
Optic Nerve	- gliosis	0	0	2	2	2	3*
Salivary gland	- amyloid	2	8	2	13*	11	16**
	- interstitial degeneration	8	2	6	1	0*	0*
Ileum	- amyloid	20	22	24	32*	28	34**
Gall bladder	- amyloid	0	0	1	0	0	5**
Pancreas	- oedema	3	9	12	8	1	0*
Spleen	- amyloid	13	13	7	10	3**	1***
Epididymides	- aspermia	10	15	20	15	26**	21*
Testes	- germinal epithelium deficit	22	23	31	29	34**	33*
	- amyloid	18	17	20	22	25	27*
Female							
Liver	- focal inflammation	1	6	5	9	5	4
	- single cell necrosis	0	0	3*	2	4*	9***
	- diffuse hepatocytic hypertrophy	5	8	6	6	7	20***
	- centriacinar hepatocytic vacuolation	0	0	0	0	4*	3*
	- periacinar hepatocytic vacuolation	0	1	0	1	17***	6**
	- amyloid	13	19	19	16	6**	3***
	- periacinar hepatocytic hypertrophy	0	0	1	0	0	3*
Skin	- ulceration	1	3	4	3	5	4
	- cellulitis	1	3	4	3	5	6
	- subcutaneous oedema	6	11	4	6	1*	1*
Heart	- amyloid	12	15	8	5*	2***	4**
	- aortic arteritis	1	0	0	0	2	4*
Kidneys	- interstitial amyloid	18	18	20	20	5***	1***
Caecum	- submucosal oedema	7	12	7	8	3*	0**
Mesenteric Lymph node							
	- lymphoid hyperplasia	3	7	2	1	2	13*
Spleen	- amyloid	11	18	13	14	1***	1***
Spinal chord	- compression	5	6	1	5	2	0*

*P<0.05, **P<0.01, ***P<0.001   (compared to pooled control K & P)

In males, the hepatic neoplasms only became evident in animals by the end of the study. A statistical evaluation of hepatocytic neoplasia, utilising non-pooled control group incidences, gave essentially very similar results though the significance of the association with treatment of hepatocytic carcinoma in the 15 and 100 ppm male groups was lost.

There were a number of other neoplasms present in animals of the 200 ppm groups that had a low incidence (1/50 or 1/49), were not statistically significant and thus considered not to be treatment-related. They included, in male mice, an oligodendroglioma of the CNS, a cutaneous

haemangiosarcoma, a urinary bladder papilloma; in females there was an adrenal medulla adenoma, ileal lymphoma, a cutaneous basal cell tumour and histiocytic sarcoma.

Table 33-9: Notable neoplastic changes in mice administered SAN 619 F in their diet for 18 months

Finding	Time of death/sacrifice	Dose (ppm)					
		0(K)	0(P)	5	15	100	200
Males							
Hepatocytic adenoma	During study	1/31	0/34	2/30	1/25	0/21	2/14
	Study termination	2/19	3/16	2/20	4/25	12/29*	10/36
	All animals	3/50	3/50	4/50	5/50	12/50**	12/50**
Hepatic carcinoma	During study	0/31	0/34	0/30	2/25	2/21	1/14
	Study termination	0/19	0/16	0/20	1/25	1/29*	0/36
	All animals	0/50	0/50	0/50	3/50*	3/50*	1/50
Females							
Hepatocytic adenoma	During study	0/33	0/27	0/26	0/30	0/14	3/23*
	Study termination	0/17	0/23	0/24	0/20	2/36	3/27
	All animals	0/50	0/50	0/50	0/50	2/50	6/50**
Hepatic carcinoma	During study	0/33	0/27	0/26	0/30	0/14	5/23**
	Study termination	0/17	0/23	0/24	0/20	0/36	2/27
	All animals	0/50	0/50	0/50	0/50	0/50	7/50***

Analysis of trends in association between incidences of all hepatocytic tumours (adenoma plus carcinoma) and dosage of cyproconazole indicate a positive association at dosages of 15, 100 and 200 ppm in male mice and at 200 ppm in female mice, as shown in Table 33-8.

However, it was noted that there was an uneven distribution of survival in this study, with better than expected survival in males given 15, 100 and 200 ppm, and in females given 100 and 200 ppm In general, animals with longer survival have a greater risk to develop tumours. This is particularly the case for males, as hepatic neoplasms only became evident in male mice by the end of the study, not in any individuals that died or were sacrificed in the interim (Table 34-2). Thus male mice with the greater life span bore the most liver tumours. (Note, for females this may not necessarily be the case as tumours were observed in female mice that died prior to terminal sacrifice). In order to assess whether the greater life span was the only cause of the greater incidences or whether there was a true effect of treatment, the notifiying company performed a Peto analysis (age-adjusted analysis for fatal, incidental and total tumours) (Table 33-9).

The results of the Peto analysis indicate an effect of difference of survival; i.e. there was a treatment-related effect in the combination of fatal and incidental tumours in male mice of the 100 ppm group and in female mice of the 200 ppm. In addition in female mice of all three categories (fatal, incidental and combined) the effect was present at the highest dosage level only. In male mice the effect (combined) of the 15 ppm group was no longer significant with Peto analysis (P=0.188) and that of the 200 ppm group just fall below the level of significance (P=0.052). In male mice of the 100 ppm group the effect was significant in the combined and incidental tumours but not in the fatal tumour group.

Table 33-10: Statistical analysis of combined liver adenoma and carcinoma in all mice (excluding satellite group) administered cyproconazole in their diet for 18 months

Parameter	Dose levels (ppm)									
	0	5	15	100	200	0	5	15	100	200
	Males					Females				
No. of animals	100	50	50	50	50	100	50	50	50	50
No. of affected animals	6	4	8	15	13	0	0	0	2	13
Statistical significance: (not adjusted for age)	###	n/s	*	***	***	###	n/s	n/s	n/s	***
Statistical significance: (age-adjusted analysis)	##	n/s	n/s	**	n/s	###	n/s	n/s	n/s	***

significant trend over affected groups, P < 0.01;   ### significant trend over affected groups, P < 0.001
*significant pairwise comparison, p < 0.05; ** significant pairwise comparison, p < 0.05; *** significant pairwise comparison, p <0.001. n/s not significant pairwise comparison, p > 0.05

Other treatment-related changes of possible toxicological significance included a slight but statistically significant increased testicular germinal epithelial deficit (22, 31, 29, 34** and 33* at 0, 5, 15, 100 and 200 ppm, respectively), aspermia (10, 20, 15, 26** and 21 at 0, 5, 15, 100 and 200 ppm, respectively) and possibly an increase in skin wounds at 100 and 200 ppm in males and an increased incidence of aortic arteritis and lymphoid hyperplasia in the mesenteric lymph modes at 200 ppm in females. The NOAEL for chronic toxicity was 15 ppm (1.84 and 2.56 mg/kg/day in males and females, respectively) based on clear evidence of hepatotoxicity at ≥ 100 ppm

Conclusion

The liver was the main target organ for cyproconazole in mice; the mouse appears to be more sensitive to the hepatotoxic effects than the rat (possibly due to differences in ratios of enzyme activity resulting in a reduced capacity of the mouse liver to metabolise and eliminate cyproconazole compared to the rat). Furthermore, male mice appeared to be more frequently affected by these hepatic changes than females. Cytotoxic changes including focal hepatocytic inflammation and single cell hepatocytic necrosis were observed in males at doses as low as 100 ppm (equivalent to 13.17 mg/kg bw/day), and were accompanied by effects associated with enzyme induction (significant increases in relative liver weight, significant increase in the incidence of hepatic accentuated lobular pattern, diffuse hepatocytic hypertrophy). Significant cytotoxic changes in the livers of female mice at 100 ppm and above (equivalent to 17.65 mg/kg bw/day) comprised centriacinar and periacinar vacuolation, as well as single cell hepatocytic necrosis. Relative and absolute liver weights were also significantly increased relative to controls in females at this dose level and there was a significant increase in the incidence of hepatic accentuated lobular pattern

Cyproconazole was carcinogenic in mice. Long-term administration of cyproconazole at doses of 100 ppm (equivalent to 13.17 mg/kg bw/day) and above caused a significant increase in the incidence of hepatocytic adenomas and carcinomas in males. In females these neoplastic changes were observed at doses of 200 ppm (equivalent to 36.30 mg/kg/day).

Thus there is an obvious species difference in the oncogenic potential of cyproconazole. Supplementary investigative studies (4.10.3 below) suggest a cytotoxic mode of action in mice by which continuous treatment with cyproconazole leads to a well-defined sequence of events, starting with a perturbation of hepatic homeostasis and resulting in degenerative lesions with subsequent liver cell proliferation leading to preneoplastic lesions and finally hepatocellular tumours. Although

several of these events were also observed in rats treated with cyproconazole, the incidence of liver tumours did not increase.

4.10.1.2   Carcinogenicity: inhalation

No data

4.10.1.3   Carcinogenicity: dermal

No data

4.10.2   Human information

No data

4.10.3   Other relevant information

Studies addressing mouse liver oncogenicity. Studies 1-4 were reported in the DAR and the additional mechanistic studies 5-7 were reported as an Addendum to the DAR (2010). In addition, 2 further *in vitro* studies were evaluated in 2014 along with a human relevance framework document. They are also briefly described in this section of the CLH report.

4.10.3.1 Four Week Liver Cell Proliferation Study in Rats and Mice

Study 1: *Cyproconazole (SAN 619 F) 4 – Week Liver Cell Proliferation Study in Rats and Mice (with Serial Sacrifices). Warren, S., Terlouw, G., Bürge, T., Dorobek, F. & Müller, F.* (1995) *Syngenta Report SAN619/5252, Sandoz Study no. 521s (BS4820). DAR B.6.8.2.1*

This 4 – week dietary study was used to determine time-dependent effects of cyproconazole (SAN 619 F) on liver cell proliferation during continuous application of the test substance through dietary admixture to rats and mice. Cyproconazole (purity 94.8%, batch no. 8507) was administered in the diet to both male Wistar rats and male CD-1 mice over a 4-week period. Rats were exposed to dietary levels of 0, 20, 350 and 1400 ppm, and mice to 0, 15, 100 and 200 ppm. On days 1, 2, 3, 4, 7, 14, 21 and 28 days, groups of 5 animals at each dosage level, were administered a timed intraperitoneal injection of 100 mg/kg bromodeoxyuridine (BrdU) which is taken up by cells actively synthesising new DNA (S-phase label), and sacrificed 2 hours later by CO_2 asphyxiation. Five control animals were sacrificed on day 0 (day of start of treatment). Mortality and clinical signs were checked twice daily (only once per day at weekends and holidays). Animals were subjected to a detailed symptom check including palpation once per week. Body weights were recorded from week-1 pre-treatment, at the start of the study, weekly thereafter and on the day of sacrifice. Mean food consumption was determined weekly and on the day of sacrifice. Blood samples were taken for clinical chemistry at necropsy. All animals underwent gross pathological examination at termination. Histological examination was limited to the liver and the BrdU-pulse labelling technique was used for the measurement of cell proliferation. A piece of the small intestine served as positive control for the liver cell proliferation assay but was not sent for evaluation.

<u>Findings</u>
Rats, 20 ppm:
Dietary consumption and food utilisation were normal. Body weight changes were normal (positive gains). There was no treatment-related mortality and no clinical signs. Blood chemistry was

unaffected. Necropsy results showed no abnormalities and liver weight did not show a statistically significant change relative to controls. Hepatocyte proliferation did not show any statistically significant difference relative to controls. Histopathology showed 1 to 3 animals per time point with trace degrees of centrilobular hepatocyte vacuolation but there was little to no difference relative to controls.

Rats, 350 ppm dose group:
Dietary consumption and food utilisation in relation to controls were reduced or impaired for week 1 only, and returned to control levels thereafter. Body weight gain was impaired relative to controls in week 1, returned to control levels thereafter. There was no treatment-related mortality and no clinical signs. Blood chemistry showed elevations in GGT activity on days 3, 4, and 7 with elevations in SDH on day 28 only. Necropsy results were normal. Liver weights increased from day 4, levelled off between days 7 to 21 and increased thereafter. Hepatocyte proliferation was not observed, there was no significant differences detected relative to controls. Histopathology showed minimal enlargement which was first detected on day 7 and remained until day 28 while trace hepatocyte vacuolation progressed from a centrilobular to a midzonal/periportal distribution.

Rats, 1400 ppm dose group:
Dietary consumption was markedly impaired during the first week but returned to normal after day 14. Body weight was adversely affected. Animals lost weight during the first week of the study, then gained weight at a slower rate thereafter until the end of the study. No mortality, piloerection was seen during the 3rd and 4th weeks of treatment. Blood chemistry showed serum ALT, AST, LDH and SDH activities peaked at day 7 and declined or showed variable results thereafter. The elevations appear to be of insufficient magnitude to indicate extensive liver cell damage. Necropsy showed accentuated lobular pattern of the liver from day 3 onwards with liver discoloration. Liver weights showed a continuous high weight from day 7 onwards until study termination. Hepatocyte proliferation was not observed. However, a statistically significant reduction in cell division was observed in high-dose animals sacrificed on days 14, 21 and 28. Histopathology showed enlargement was first detected on day 2 and progressed to moderate on day 7, remaining at this degree until day 28. Moderate hepatocyte vacuolation progressed from a centrilobular to a midzonal/periportal distribution.

Mice, *15 ppm dose group:*
Dietary consumption and food utilisation were unaffected. Body weight gain was very slightly reduced during the first week and normal thereafter. There was no mortality and no clinical signs. Blood chemistry was normal. Necropsy results showed a low incidence of accentuated lobular pattern of the liver and discoloration of the liver. Liver weight did not show a statistically significant change relative to controls. Hepatocyte proliferation showed a transient, distinct, early increase with statistical significance, for day 3 only. Histopathology showed minimal enlargement was first detected on day 4, and remained at this degree until day 28. Hepatocyte vacuolation was evident in a few mice after day 7.

Mice, 100 ppm dose group:
Dietary consumption and food utilisation were unaffected. Body weight gain was very slightly reduced during the first week, and normal thereafter. There was no mortality and no clinical signs. Blood chemistry was unaffected. Necropsy results showed an increased incidence of accentuated lobular pattern of the liver and only 1 animal at day 28 showed discoloration of the liver. Liver weight increased moderately and appeared to peak at about day 7 and declined thereafter slightly with time. Hepatocyte proliferation showed a transient, distinct, early increase with statistical significance, compared to control for day 3 only. Histopathology showed minimal enlargement which was first detected on day 2 and progressed to moderate on day 7, remaining at this degree until day 28. Hepatocyte vacuolation was progressively more evident from day 2 onwards.

Mice, 200 ppm dose group:
Dietary consumption and food utilisation were unaffected. Body weight gain was very slightly reduced during the first week and normal thereafter. There was no mortality and no clinical signs. Blood chemistry

showed serum elevations of ALP, AST and SDH which generally peaked around day 7 (day 4 in the case of alkaline phosphatase) but appear to be of insufficient magnitude to indicate extensive liver cell damage. Necropsy results showed an increased incidence of accentuated lobular pattern of the liver and only 1 animal at day 28 showed discoloration of the liver. Liver weight was increased moderately and appeared to peak at about day 7 and declined thereafter slightly with time. Hepatocyte proliferation showed a transient, distinct, early increase with statistical significance, compared to control for day 3 only. Histopathology showed minimal enlargement which was first detected on day 2; progressed to moderate on day 3 and remained at this degree until day 28. Hepatocyte vacuolation was progressively more evident from day 2 onwards.

Conclusions

There was no mortality during the study; all animals survived through to their scheduled sacrifice time. Treatment-related findings at necropsy were confined to the liver for both species. No other organ systems appeared to be adversely affected even with high doses of Cyproconazole. High levels of dietary cyproconazole caused moderate body weight alterations and some hepatotoxicity in both rats and mice. The marked increases in liver weights appear to be a result of hepatocyte hypertrophy rather than hyperplasia. These changes were accompanied histopathologically by centrilobular hepatocyte enlargement and vacuolation in both species. Blood chemistry measurements were inconclusive but did indicate an adaptive response to a chemical insult at high dose levels. Statistical significance must be treated with caution, as it does not necessarily indicate biological significance; in some cases inappropriate statistical tests were employed or there were insufficient sample numbers to arrive at a conclusion. Clear species differences were also observed in relation to hepatocyte proliferation (a transient, early increase in mice, but a later decrease below control levels in the rat - there was however no clear evidence of increased cell proliferation in rats), and the distribution of hepatocytes exhibiting vacuolation with high dose treatments.

In the rat study, the low dose group of 20 ppm (1.5 mg/kg/day) was found to be the no-effect level, In the mouse study a no-effect level could not be established and 15 ppm (2.2 mg/kg/day) was set as a low-effect level for hepatic cell proliferation, enlargement and vacuolation.

4.10.3.2 Histopathological Evaluation of the Effects of Cyproconazole and Propiconazole on the Liver of Male Mice

Study 2: *Comparative Histopathologic Evaluation of the Effects of Cyproconazole and Propiconazole on the Liver of Male Mice. (re-evaluation of slides of Warren et al., 1995, Syngenta Report SAN619/5252 and Weber, 1999, Novartis Report No. CB 97/23). DAR B.6.8.2.1.2*

This study is a histopathological re-evaluation and is intended to evaluate the histopathological effects of subchronic treatment with cyproconazole and propiconazole on the liver of male mice. Haematoxylin & eosin (H&E) stained liver sections from previous studies of comparable protocol were re-examined by a single pathologist to ensure the use of uniform grading criteria and terminology. The slides evaluated were taken from the following studies: (1.), a cell proliferation study with cyproconazole in mouse (*Warren et al., 1995,* Study No. 521S, Syngenta Report SAN619/5252); and (2.), a similar study performed with propiconazole which had phenobarbitol included as a positive control (*Weber, E., 1999*, Novartis Study Report No. CB 97/23, File No. CGA64250/4200). Only the phenobarbital data was presented as a comparator.

Study protocol:
(1). Cyproconazole.

Study:	*Warren et al., 1995,* Study No. 521S
Doses:	0, 15, 100, and 200 ppm.
Treatment periods:	1, 2, 3, 4, 7, 14, 21, and 28 days.
Animals:	5 animals per dose and time point.

(2). Propiconazole (including positive control: phenobarbital).
  Study: *Weber E., 1999,* Study Report No. CB 97/23
  Doses: 0, 850, and 2500 ppm (phenobarbital: 850 ppm).
  Treatment periods: 1, 2, 3, 4, 7, 14, 28 and 60 days.
  Animals: 5 animals per dose and time point.

Both studies originally investigated the effect of the specific triazole treatment on hepatic cell proliferation (as measured using the BrdU labelling assay), and also included H&E stained liver sections. The high number of serial sacrifices allows a potentially detailed assessment of the timely occurrence of histopathological changes in the liver. In addition, the propiconazole study included phenobarbital treated groups, which served as positive controls for liver growth inducing compounds. The following aspects were compared for cyproconazole and Phenobarbital; liver weights; degree of hepatocellular hypertrophy; hepatocellular mitotic activity; degree of hepatocellular necrosis; hepatocellular vacuolation.

Findings
On subchronic administration to mice, propiconazole and cyproconazole in high doses, caused similar patterns of histological change in the liver. There were no significant qualitative differences between the two triazoles and quantitative differences generally reflected the different degrees of induced hepatomegaly. In addition, the overall picture resembles that observed with phenobarbital. However there were two qualitative differences between cyproconazole (and propiconazole), and phenobarbital worth noting:

   (1.) Hypertrophic hepatocytes were distinctly or more clearly localised to the centrilobular/midzonal regions with phenobarbital treatment, cyproconazole resulted in a less-defined distribution of affected hepatocytes,

   (2.) Phenobarbital at 850 ppm was not seen to induce centrilobular cytoplasmic vacuolation in contrast to cyproconazole treatments where varying degrees of centrilobular hepatocyte vacuolation was evident, becoming more frequent and severe with increasing dose and time on treatment.

Increased liver weight is a common observation upon subchronic treatment of rodents with Phenobarbital. Together with propiconazole and cyproconazole, these compounds induced liver growth in a generally dose-dependent manner in the following order: propiconazole 2500 ppm > phenobarbital 850 ppm > propiconazole 850 ppm ≈ cyproconazole 200 ppm > cyproconazole 100 ppm > cyproconazole 15 ppm. Cyproconazole displayed the lowest degree of induced hepatomegaly presumably because of its considerably lower administered dose.

The observed hepatomegaly after treatment is presumed to be a consequence of both hypertrophy and increased proliferation of hepatocytes. Hepatocellular hypertrophy is frequently seen in the liver following exposure to agents that cause hepatic enzyme induction (such as phenobarbital). Hypertrophy was less pronounced in cyproconazole than in phenobarbital-treated animals, presumably due to its lower administered dose relative to the other compounds. Hypertrophic hepatocytes were mainly found in the centrilobular and midzonal lobular compartments but also throughout the whole lobular compartment (except for phenobarbital which was more distinctly localised to the centrilobular/midzonal regions). The severity and frequency of occurrence was clearly both dose- and time-dependent.

With phenobarbital, the time- and dose-relationships of the mitotic activity (i.e. abundance of mitotic figures in H&E-stained sections) are similar to those of the bromodeoxyuridine labelling index (data not shown in this report). In line with the strong induction of cell proliferation observed with phenobarbital (up to 95-fold over controls) (data not shown), and the relatively lower induction of cell proliferation with cyproconazole (up to 12-fold over controls) (*Warren et al. 1995*), considerably increased mitotic activity was found with

phenobarbital on treatment days 2 and 3, but not with cyproconazole. This lack of mitotic activity in cyproconazole-treated animals could possibly be due to the use of the less sensitive technique of H&E-labelled sections for observance of mitotic figures versus the more sensitive technique of immunostaining for BrdU labelled cells. This suggestion in the original report does not however negate the fact that cyprocoazole is not as effective in inducing mitotic activity as propiconazole. The effect or lack of effect may also be due to the differences in administered dosages as the levels of cyroconazole were far less than propiconazole in the original studies.

The extent of induced hepatocellular necrosis roughly corresponded to the induction of heptocellular hypertrophy. Minimal necrosis was evident in all cyproconazole–treated groups at a low incidence (generally 1 – 2 animals per group), at all-time points, including the low dose 15 ppm groups.

Centrilobular cytoplasmic vacuolation incidence and severity were both dose- and time-dependent for cyproconazole and little to no occurrence with phenobarbital treatment (1 animal amongst all the groups). Cyproconazole was a stronger inducer of this effect than propiconazole. It was speculated that the higher level of cellular hypertrophy and proliferation with propiconazole treatment may mask the true occurrence or suppress vacuolation in propiconazole-treated animals, but no evidence was included to support this suggestion. In conclusion:

(1.) Treatment of male mice with cyproconazole (15, 100, or 200 ppm) or propiconazole (850 or 2500 ppm) for up to 28 days caused a similar pattern of histopathological changes in the liver.
(2.) no significant qualitative differences between the two triazoles (there were quantitative differences due to dose).
(3.) cyproconazole effects resemble those observed with phenobarbital.

4.10.3.3   Enzyme Induction in the Rat and Mouse Liver.

Study 3: *CYPROCONAZOLE: Investigations of Enzyme Induction in the Rat and Mouse Liver. Dorobek, F. & Müller, F., (July 1995). Syngenta File No. SAN 619/5207, Sandoz Study No. 558 S. DAR B.6.8.2.1.3*

The objective of this study was to conduct a comparitive liver enzyme induction study in rats and mice, in order to detect the extent of enzyme induction or inhibition by treatment with cyproconazole. Samples of livers from rats and mice were obtained from the Cell Proliferation Study, previously described (*Study 2 above*). For this investigation liver samples from male Han-Wistar rats and male CD-1 mice treated with cyproconazole, contained in the diet, for 4 and 21 days at the dose levels of 1400 and 200 ppm, respectively, and liver samples from the corresponding controls, were used (5 animals per dose, species and time point. The following parameters and enzyme activities were investigated:

Phase I related parameters (microsomal):
- total cytochrome P-450 content   (Cyt P-450)
- NADPH-cytochrome P-450 reductase   (NCPR)
- ethoxyresorufin-0-deethylase   (EROD, a CYP1A related activity)
- pentoxyresorufin-0-dealkylase   (PROD, a CYP2B related activity)

Phase II related parameters (microsomal & cytosolic) :
- total glutathione content   (GSH),
- glutathione S-transferase   (GST),
- UDP-glucuronyl transferase   (UDPGT).

Findings:

Administration of cyproconazole *in vivo* has shown that the liver is the main target organ in both rats and mice. The present study clearly shows that cyproconazole has an inductive effect on the phase I enzymes, particularly in the rat with respect to CYP2B (PROD) which is greatly induced, having a specific activity 26 to 36-fold higher than in mice.

The classical route of xenobiotic metabolism is activation (or inactivation) of the compound through the actions of the phase I enzymes of the endoplasmic reticulum (involving oxidation, reduction, dealkylation and hydrolysis reactions) followed by conjugation reactions performed by several members of the phase II detoxification enzymes (involving glucuronidation, sulphation, acetylation or addition of glutathione).

Effects on Phase I Metabolism: Cyproconazole induces enzymes of phase I and II metabolism by factors of approximately 1.5 to 4.0 times that of controls in both rats and mice. However, it also proves to be a particularly strong inducer of the CYP2B family of cytochrome P450 isozymes in rats (determined as PROD activity) but much less so in mice. In mice, CYP1A mediated activity remains greater than CYP2B activity (by about 10-fold) even though there is a higher level of induction of CYP2B with cyproconazole treatment. There are also differences in other enzyme systems, but the ratio of the CYP2B to CYP1A activities in both species would probably have the greatest influence on the pattern of the resulting metabolites of cyproconazole.

A significant difference was demonstrated in induction of CYP2B type activity between rats and mice. No adequate explanation was provided in the report and no further experiments were undertaken to investigate the effect. The difference may be due to the presence of an enzyme inhibitor. Since all the other enzyme activities were broadly similar between rat and mouse the authors should have addressed this question.

The other phase I enzymes examined included total cytochrome P450 and NADPH cytochrome P450 reductase (NCPR). Changes in the total cytochrome P450 content could have an effect on the metabolic distribution and pattern since the excess NCPR activity (by about 2 orders of magnitude, that feeds into the P450 cycle ensures that the P450 system is operating at maximal rate regardless of its induction level. Conversely, changes in NCPR activity would be expected to have little or no effect under this system, as it is already present in excess.

Effects on Phase II Metabolism: Cyproconazole does not seem to be as effective in inducing enzymes of phase II metabolism with the highest induction occurring by day 21 for rat. A clear species difference arises from the results; rats show higher UDPGT and GST induction by day 21 but with lower specific activity than those found in mice. Cyproconazole appears to inhibit UDPGT induction in mice at both time points. GSH (substrate for GST activity) levels are variable being higher in mice initially but decreasing below that of rat by day 21.

The capacity of the rat liver to metabolise and eliminate cyproconazole appears greater than that of the mouse. Rat liver responds to cyproconazole treatment by greater induction of many of the enzymes associated with phase I and II detoxification pathways. However, mice often had greater overall enzyme activity for NCPR, EROD (CYP1A), GST and UDPGT enzymes. In addition, the different ratios of enzyme activities, particularly with regard to CYP1A and CYP2B could potentially result in a different ratio or pattern of metabolites between rats and mice. If the CYP1A isozymes metabolise xenobiotics to reactive intermediates with functional groups that could sterically hinder further metabolism by phase II systems (as suggested in the original report and by implication suggesting a greater toxic response), then the dominance of the CYP1A activity over the CYP2B activity in mice could help to understand the increased cyproconazole sensitivity in

mice and the lack of effect from a more active phase II detoxification system. However, the large discrepancy in CYP2B activity (PROD) was not addressed in this study.

4.10.3.4   Enzyme Activity in the Male and Female Mouse Liver

Study 4: SAN 619 A (Cyproconazole): Effects on Biochemical Parameters in the Liver following Dietary Administration to Male and Female Mice. Trendelenberg, C., February 2001. Syngenta File No. SAN 619/7076, Report No. CB 00/13. (DAR B.6.8.2.1.4)

This study was designed to describe liver enzyme induction (both biochemically and immunochemically) in male and female CD-1 mice following dietary administration of cyproconazole (SAN 619 A). Phenobarbital was also used as the reference compound for cytochrome P450 isoenzyme induction. Groups of 5/sex CD-1 mice were randomly assigned and treated for 14 consecutive days with cyproconazole at dietary concentrations of 0, 50, 100 and 200 ppm (corresponding to mean daily doses of 0, 9.0, 16.7 and 24.8 mg/kg bw for males and 0, 12.7, 21.5 and 29.5 mg/kg bw for females, respectively). Additional groups of 5 male and 5 female mice were treated with the reference compound phenobarbital, a known, potent model enzyme inducer in rodent species, at 850 ppm corresponding to a mean daily dose of 125.7 mg/kg bw for males and 152.9 mg/kg body weight for females, respectively. Body weight and food consumption were recorded pre-treatment and daily for 14 days. Gross necropsy was carried out on sacrifice. The liver was taken for histological examination and samples for biochemical analysis as follows:

Table 34-1:   Summary of parameters investigated

Biochemical and Immunochemical Parameters Investigated in Male and Female Mice	
Parameter:	**Activity typified by:**
100 x g supernatant protein	
Total microsomal protein	
Total cytosolic protein	
Total cytochrome P450 content	
Microsomal 7-methoxyresorufin O-demethylase	CYP1A 1/2
Microsomal 7-ethoxyresorufin O-deethylase	CYP1A 1/2
Microsomal 7-pentoxyresorufin O-dealkylase*	CYP2B
Microsomal 7-benzyloxyresorufin O-dealkylase*	CYP2B
Microsomal coumarin 7-hydroxylase	CYP2A6
Microsomal lauric acid 11-hydroxylase	CYP2E
Microsomal lauric acid 12-hydroxylase	CYP4A
Total microsomal testosterone oxidation	
Microsomal testosterone 1α-hydroxylase	
Microsomal testosterone 2α-hydroxylase	CYP2C11
Microsomal testosterone 2β-hydroxylase	CYP3A2
Microsomal testosterone 6α-hydroxylase	
Microsomal testosterone 6β-hydroxylase	CYP3A2
Microsomal testosterone 7α-hydroxylase	CYP2A1
Microsomal testosterone 15α-hydroxylase	CYP2A2
Microsomal testosterone 15β-hydroxylase	CYP3A
Microsomal testosterone 16α-hydroxylase	CYP2C11
Microsomal testosterone 16β-hydroxylase	CYP2B 1/2
Microsomal 17β-hydroxysteroid dehydrogenase (androstenedione formation from testosterone)	
Microsomal epoxide hydrolase	
Microsomal UDP-glucuronosyl transferase	
Cytosolic glutathione S-transferase (GST)	
Peroxisomal fatty acid β-oxidation	

Immunoblot analysis of CYP1A isoenzymes	
Immunoblot analysis of CYP2B isoenzymes, band 1	
Immunoblot analysis of CYP2B isoenzymes, band 2	
Immunoblot analysis of CYP3A isoenzymes	
Immunoblot analysis of CYP4A isoenzymes	

* For all animals, PROD activity was determined at 3 different concentrations of microsomal protein equivalent to 0.228, 0.683 and 2.05 mg wet liver equivalents per assay. Similarly, for all animals, BROD activity determined at 3 different concentrations of microsomal protein equivalent to 0.046, 0.137 and 0.410 mg wet liver equivalents per assay.

Findings:

Treatment of male and female CD-1 mice with cyproconazole and phenobarbital caused hepatomegaly, which was accompanied by an induction of the hepatic microsomal cytochrome P450 system and phase II xenobiotic metabolising enzymes. The induction profile was very similar with both compounds and comprised:

1. Induction of cytochrome P450 isoenzymes of subfamily CYP2A as indicated by increased COH and testosterone 15α-hydroxylase activities,

2. Induction of cytochrome P450 isoenzymes of subfamily CYP2B as indicated by increased protein contents of immunochemically detectable cytochrome P450 isoenzymes of this gene subfamily and, with phenobarbitone, by a clear induction of PROD and BROD activities,

3. Induction of cytochrome P450 isoenzymes of subfamily CYP3A as indicated by increased protein contents of immunochemically detectable cytochrome P450 isoenzymes of this gene subfamily and by an increased testosterone 6ß-hydroxylase activity,

4. Induction of the phase II enzymes mEH, microsomal UDPGT and cytosolic GST.

A marginal induction of PROD activity upon treatment with cyproconazole (2 to 3-fold at 200 ppm) was noted. This enzyme activity is a key diagnostic marker for mouse liver CYP2B which was shown initially by immunoblot analysis to be strongly induced by phenobarbital (19 to 35-fold in males and females respectively) as well as by cyproconazole (12 to 31-fold at 200 ppm in males and females respectively). A similar effect was observed for BROD, which represents another monooxygenase activity known to be efficiently, albeit not exclusively, catalysed by cytochrome P450 isoenzymes from subfamily CYP2B.

Additional investigations of these enzyme activities confirmed the presence of an inhibitory factor in microsomal fractions from cyproconazole-treated animals, which specifically inhibited monooxygenase activities catalysed by cytochrome P450 isoenzymes of subfamily CYP2B. The heat-inactivated preparations of microsomal fractions would preferentially denature large proteins such as enzymes but have far less of an effect on smaller molecular weight components such as peptides. Cyproconazole is therefore, like phenobarbital, a strong inducer of CYP2B isoenzymes, as demonstrated by immunochemical analysis. However, the low molecular weight inhibitory factor present in microsomes from cyproconazole-treated animals does not allow the accurate measurement of CYP2B-dependent activities in these microsomal fractions.

Inhibition of CYP2B type activity is possibly more complex than the explanation submitted in the company report. There is little reference to the reduction in PROD and BROD activities in phenobarbital treated animals (particularly females) with the highest concentrations of microsomal protein. This appears to be a distinct effect separate from that of a specific inhibitor as seen in the cyproconazole-treated animals. It is proposed that the second mechanism may be related to product

inhibition or lack of substrate availability as increasing amounts of enzyme would normally predict an increase in activity subject to no interferences such as those mentioned.

In nearly all cases the results indicate that the metabolic potential in female animals is much greater than in the males (through increased basal levels of enzyme activity and a greater response to induction by xenobiotics), and this would probably account for the increased sensitivity of males to cyproconazole and phenobarbital exposure.

Overall, dietary treatment of male and female CD-1 mice with 50, 100 and 200 ppm cyproconazole caused a pronounced hepatomegaly. Cyproconazole was found to be a dose-dependent, strong phenobarbital-type inducer of xenobiotic-metabolising enzymes in rat and mouse liver. In the mouse this was comprised of an induction of cytochrome P450 isoenzymes of subfamily CYP2A, CYP2B and CYP3A and the phase II enzymes microsomal epoxide hydrolase, microsomal UDP-glucuronosyltransferase and cytosolic glutathione S-transferase. Likewise, a mode of action of cyproconazole as a polycyclic aromatic hydrocarbon- or peroxisome-type inducer can be excluded as cytochrome P450 isoenzymes of subfamily CYP1A and CYP4A were only slightly induced if at all.

4.10.3.5   14 Day Mouse Strain Dietary Study with Cyproconazole

Study 5.   Cyproconazole (SAN619) And Phenobarbital: 14 Day Dietary Study for the Evaluation of Liver Effects in Three Strains of Mice. Milburn G, (2006a).   Syngenta Report Number: XM7470-TEC. DAR Addendum B.6.8.2.1

The subchronic and oncogenicity studies with cyproconazole (CCZ) were conducted in CD-1 mice, but knockout mice for the CAR receptor are not available in this strain. The primary purpose of this study was to produce sufficient data to allow an alternate male mouse strain (C57BL/6J or C3H/HeNClrBR) to be selected as a surrogate for CD-1 mice in subsequent constitutive androstane receptor (CAR)-knockout mouse experiments. Key criteria included liver toxicity responses to CCZ similar to that seen in the CD-1 mice studies previously conducted. For reasons of comparison, phenobarbital (PB) was included in this study. PB is known to cause a large variety of effects in the rodent liver which are mediated by the CAR receptor. Liver effects (weight/clinical chemistry/histology) and biochemical analysis were compared between the strains.

Effects on the liver involving hypertrophy, fat vacuolation, a concurrent decrease in plasma cholesterol and increased liver weight appear beginning 2 days after treatment. By 7 days, single cell necrosis is also observed, with accompanying changes in clinical chemistry markers of liver damage. Some differences in the details of how CCZ treatment affected liver parameters compared to PB were observed.   Hypertrophy was primarily centrilobular with PB but centrilobular/panlobular with CCZ. Fat vacuolation and decreased cholesterol were much more pronounced with CCZ than with PB.

Table 34-2:   Mouse strain comparison. Day 15 data (% of controls unless otherwise stated)

Observation	CD-1 (mg/kg bw)			C57 (mg/kg bw)			C3H (mg/kg bw)		
	38.3	**90.7**	**167.4**	**45.3**	**110.2**	**189.9**	**61.2**	**131.2**	**239.6**
	(200) CCZ	(450) CCZ	(850) PB	(200) CCZ	(450) CCZ	(850) PB	(200) CCZ	(450) CCZ	(850) PB
Dose relative to CD-1	100	100	100	118	121	113	160	145	143
plasma cholesterol	61.4	44.3	87.4	43.4	37.0	87.7	45.6	39.2	86.8
plasma triglycerides	92.1	114	102	90.4	76.5	101	148	240	161
plasma bilirubin	161	202	145	127	109	95.6	223	388	223
plasma ALP	128	150	105	148	166	97.6	125	154	110
plasma GluDH	167	265	164	217	983	129	187	329	132
plasma ALT	173	407	239	348	2974	147	160	251	197
plasma AST	100	152	100	127	643	101	149	185	101
liver weight	123	160	135	120	131	139	124	149	134
Ki67 labeling index	57.1	236	121	--	--	--	80.8	425	137
Mdm2 mRNA levels*	--	--	--	--	--	--	1.04	1.36	1.01
Gadd45β mRNA levels*	--	--	--	--	--	--	8.20	27.7	21.4
Cyp2b10 mRNA levels*	--	--	--	--	--	--	280	446	212
Cyp 2b protein levels*									
(upper band)*	4.3[§]	5.6[§]	5.9[§]	3.9[§]	4.1[§]	3.8[§]	4.4[§]	4.6[§]	5.4[§]
(lower band)*	1.2	1.4	1.4	1.3	1.3	1.2	1.1	1.0	1.2
BROD relative activity	492	1229	3318	307	613	2983	391	414	2230
PROD relative activity	185	314	830	177	238	4608	221	223	5180
Coumarin 7-hydroxylase (CYP 2a5 activity)	--	--	--	--	--	--	376	738	304

* levels expressed as relative difference to controls
[§] upper band compared to low levels found in the respective controls

The findings indicate that the use of alternative strains for the investigation of the role of the CAR receptor is acceptable though the results for the C57 strain indicate it may be more sensitive to the cytotoxic effects of CCZ at high doses. Even though the C3H strain received a higher dose of test material relative to the CD-1 strain, it can be seen that similar type responses are observed and it is therefore a suitable surrogate for investigating CAR-mediated events.

4.10.3.6   7 Day Dietary Study with Cyproconazole in Wild Type vs CAR null mice.

Study 6: Milburn G, (2006b).   Exposure of Wild-Type and CAR null C3H Male Mice via the Oral (Dietary) Route for 7 Days. Syngenta Report Number: XM7573-TEC. DAR Addendum B6.8.2.2

Constitutive androstane receptor (CAR)-dependent pathways are thought to be responsible for the non-genotoxic effects of phenobarbital (PB) in promoting hepatic tumours in mice following long-term dosing studies. This mode of action for phenobarbital is thought to be of little relevance to man. It was proposed that the action of cyproconazole (CCZ) in the mouse 18 month study is a consequence of CAR receptor activation as seen for phenobarbital, and its subsequent downstream effects on other gene targets that eventually lead to the higher incidence of liver tumours seen with high doses of CCZ. In these previous studies CD-1 mice (males and females) had liver tumors and similar pre-neoplastic histopathology findings in the liver after prolonged dietary exposure to CCZ. Male mice were more sensitive and showed the greatest effects.

Cyproconazole (CCZ) was administered in the diet to both wild type C3H/HeNClr mice (the normal population) and gene knockout animals that lack a functional CAR nuclear receptor, leading to altered gene expression for CAR-responsive targets. This strain has been shown to give a liver response to CCZ similar to that seen in CD-1 mice and so is a suitable surrogate. The present study illustrates the difference in responses to CCZ treatment between the two animal populations. It provides clear evidence for the involvement of the CAR receptor system in mediating the biological responses to CCZ in mice. This study does not address similarity to phenobarbital responses nor is it a chronic, long term investigation.

The CCZ dose received was 38.6 mg/kg for wild type mice treated with a nominal 200 ppm, 34.0 mg/kg for CAR null mice treated with 200 ppm, 71.5 mg/kg for wild type mice treated with 450 ppm and 54.7 mg/kg for CAR null mice treated with 450 ppm. CAR null groups contained a variable number of animals per group (n = 3 to 5 mice/group) while there were 5 animals per group amongst the wild-type mice groups. There were no treatment-related clinical observations. In wild type mice treated with 450 ppm CCZ there were slightly reduced body weights. Many other effects were recorded due to CCZ treatment. The CAR-null genotype negates some of the effects of CCZ implying that initial activation of the CAR receptor is required for some of the effects promoted by CCZ exposure. The increases in liver weight and release of ALT into plasma are radicaly reduced in comparison to CCZ treatment of wild type mice but some other responses are similar implying that there are further events downstream of CAR activation that may be at work and utilise other pathways not wholly dependent on CAR (see table below).

Table 34-3:   CCZ effects in Wild type and CAR null genotypes

Observation	*Wild type (mg/kg bw)*			*CAR-null (mg/kg bw)*		
	0	*38.6*	*71.5*	*0*	*34.0*	*54.7*
		(200ppm)	*(450ppm)*		*(200ppm)*	*(450ppm)*
plasma cholesterol	100%	41%	29%	100%	103%	73%
plasma triglycerides	100%	79%	41%	100%	101%	39%
plasma ALP	100%	130%	154%	100%	112%	147%
plasma ALT	100%	196%	1004%	100%	94.9%	52.6%
plasma AST	100%	192%	461%	100%	147%	173%
liver weight	100%	129%	135%	100%	107%	100%
Ki67 labeling index	100%	209%	495%	100%	142%	189%
Mdm2 mRNA levels	1	0.93	1	1	0.93	1.23
Gadd45β mRNA levels	1	2.46	6.06	1	1	4.29
Cyp2b10 mRNA levels	1	147	294	1	2.5	4
Cyp 2b relative protein levels						
(upper band)	0	2.3	1.6	0	0	0
(lower band)	1	2.6	1.6	1	1.2	1
Coumarin 7-hydroxylase (CYP 2a enzyme activity)	100%	849%	1135%	100%	472%	458%

CAR null mice exhibited some effects on the hepatic system following cyproconazole exposure. In summary:

1. increased coumarin 7 hydroxylase activity,
2. no effect on Cyp2b expression,
3. Mdm2 and Gadd45 expression levels were similar to wild type responses,
4. Reduction in plasma lipids with high dose CCZ but not at 200ppm,
5. slight increases in plasma AST,
6. slight increases in Ki67 labeling index.

There is strong support for an agonistic interaction between CCZ and the CAR receptor. There is up regulation of some of the genes known to be targets of the CAR regulatory system (such as CYP2b), increases in liver weight and hypertrophy, and minimal/slight hepatocyte damage. However, differences are also apparent relative to those effects expected from CAR activation or phenobarbital (PB) exposure (little change in Mdm2 mRNA levels, increased cell proliferation independent of CAR, increased CYP 2a activity independent of CAR). Not all the effects of CCZ are wholly mediated by CAR activation. Other events downstream of this system and CAR-independent events influenced by CCZ exposure such as PXR crosstalk may also be operating and perhaps account for some of the responses in the CAR null mice.

4.10.3.7   3 and 7 Day Dietary Study with Phenobarbital in Wild Type vs CAR null mice

Study 7: Milburn G, (2006c). Phenobarbital: Exposure of Wild-type and CAR null C3H Male Mice via the Oral (Dietary) Route for 3 and 7 days. Syngenta Report Number: XM7584-TEC-R2. DAR Addendum B6.8.2.3

This study illustrates that CAR plays a significant role in the liver effects of phenobarbital (PB) in mice by comparing the responses in wild-type and CAR-null mice. Hence, the effects of phenobarbital on liver histopathology and related biochemical events in wild type C3H/HeNClr mice and constitutive androstane receptor null (CAR null) mice derived from the same background strain were investigated.

Male mice were treated with phenobarbital admixed to the diet at a concentration of 850 ppm for 3 or 7 consecutive days. In the 3-day and 7-day treatment groups, the phenobarbital dose received was 140.4 and 127.7 mg/kg for wild type mice and 117.1 and 130.5 mg/kg for CAR null mice, respectively.

Table 34-4:   PB effects in Wild type and CAR null genotypes

Observation	Wild type (mg/kg bw)			CAR-null (mg/kg bw)		
	0	140.4	127.7	0	117.1	130.5
		(850ppm) day 3	(850ppm) day 7		(850ppm) day 3	(850ppm) day 7
plasma cholesterol	100%	81%	84%	100%	86%	88%
plasma triglycerides	100%	73%	145%	100%	103%	108%
plasma ALP	100%	108%	116%	100%	107%	104%
plasma ALT	100%	251%	--%	100%	140%	--%
plasma AST	100%	187%	--%	100%	166%	--%
liver weight	100%	137%	134%	100%	88%	97%
Oil Red O (% area stained)	0.28	7.84	0.49	0.92	0.96	0.72
Ki67 labeling index	100%	852%	306%	100%	36%	130%
Mdm2 mRNA levels	1	1.07	1.07	1	1.23	1.23
Gadd45 mRNA levels	1	6.96	4.92	1	2.00	2.14
Cyp2b10 mRNA levels	1	239	194	1	0.71	5.28
Cyp 2b relative protein levels						
(upper band)	0	1.7	1.3	0	0	0
(lower band)	1	1.6	1.3	1	1.2	0.9
BROD relative activity	1	19.2	43.8	1	1	1
PROD relative activity	1	73.1	153	1	1.6	0.9
Coumarin 7-hydroxylase (CYP 2a5 enzyme activity)	100%	334%	624%	100%	100%	105%

There were no treatment-related clinical observations. In wild type and CAR-null mice treated with 850 ppm PB there was little to no effect on body weights. Many other effects were recorded due to

PB treatment. The CAR-null genotype negates many of the effects of PB implying that initial activation of the CAR receptor is required for the effects promoted by PB exposure.

Conclusion: As can be seen from the table above, the administration of phenobarbital for 3 or 7 days to wild type and CAR-null mice at a dietary concentration of 850 ppm was associated with major differences in the responses of the liver between the two strains. These data confirm the potency of phenobarbital as an inducer of murine hepatic Cyp2b. They also confirm that this induction is confined to wild type mice, as only minor changes were observed in CAR null mice.

4.10.3.8   *In Vitro* Mouse Hepatocyte Cell Culture

Study 8:   Elcomb, B., (2011a).   Cyproconazole – Cytochrome P450 2b, 3a and DNA-Synthesis Induction in Cultured Male Mouse Hepatocytes. Syngenta, CXR1093.   New in vitro mouse hepatocyte assay.

This study investigated the ability of cyproconazole to induce cytochrome P450 2b (Cyp2b) transcript levels, Cyp3a transcript levels and potential changes in cell proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) in isolated male mouse hepatocyte cultures. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) were included as known positive controls for induction of Cyp2b/3a transcript levels and cell proliferation, respectively.

Hepatocytes were exposed to cyproconazole at 6 concentrations (0.2, 1, 5, 25, 125 and 500μM) for 96 hours. PB was used at 3 concentrations (10, 100 and 1000 μM), for 96 hours. Vehicle was 0.5% (v/v) dimethyl sulfoxide for 96 hours. Cell toxicity/viability was quantified based on the amount of cellular adenosine-5'-triphosphate (ATP), low amounts relative to vehicle controls indicate low viability or increased cytotoxicity.

A few key points arise from this investigation:

(1.)   Treatment with 500μM cyproconazole resulted in significant cytotoxicity, with intracellular ATP levels being reduced to 2% of control. There was no measurable RNA isolated from hepatocytes treated with 500 μM cyproconazole.

(2.)   There was no cytotoxicity associated with PB.

(3.)   A small dose-dependent increase in the expression of Cyp2b10 was observed following treatment of hepatocytes with PB resulting in a 2.0- to 2.8-fold induction of Cyp2b10 mRNA over the vehicle controls.

(4.)   Cyproconazole at concentrations from 0.2 μM to 25 μM, induced increased CYP2B6 mRNA expression relative to the DMSO control. Cyp2b10 mRNA expression levels were increased by as much as 2.7-fold.

(5.)   PB induced a small increase (1.3-fold versus control) in the expression of Cyp3a11 mRNA following treatment of hepatocytes with the highest dose of 1000 μM.

(6.)   Cyproconazole induced Cyp3a11 mRNA expression after treatment of hepatocytes at concentrations from 0.2 μM to 25 μM (1 - 1.8-fold versus control) in a dose-dependent manner. Exposures greater than 25 μM resulted in lower levels of induction for Cyp3a11 mRNA expression.

(7.) Treatment with cyproconazole resulted in a maximum dose-dependent increase in replicative DNA synthesis of 3.9-fold versus control.

(8.) Treatment with PB resulted in an increase in replicative DNA synthesis of 2-fold versus control at 100 μM.

(9.) Treatment with EGF resulted in a statistically significant increase in replicative DNA synthesis of 10.2-fold versus control.

Increased transcript levels of Cyp2b10, Cyp3a11 and increased cell proliferation following PB treatment demonstrate the sensitivity of the cultured male mouse hepatocyte assay. Treatment with cyproconazole resulted in the induction of both Cyp2b10 and Cyp3a1 mRNA transcript levels along with a marked increase in cell proliferation.

Conclusion: When administered to rats and mice, compounds such as phenobarbital elicit hepatomegaly that is characterised by increased replicative DNA synthesis, cell proliferation, smooth endoplastic reticulum (SER) proliferation and enzyme induction, especially the induction of Cyp2b via the activated nuclear receptor CAR (or Cyp3a via the activated nuclear receptor PXR).

Based on lowered intracellular ATP concentrations, 500 μM cyproconazole is considered to be cytotoxic to mouse hepatocytes. As a result of significant cytotoxicity, replicative DNA synthesis measurements were not made following treatment at the high dose.

The situation at 125 μM cyproconazole appears more complex. This dose level correlates with the highest level of intracellular ATP and the highest level of DNA synthesis. However these changes occur with the lowest expression levels for both Cyp2b10 and Cyp3a11 mRNA. The degree of involvement of CAR and PXR in the overall process is unclear at this dose level.

Cyp2b10 and Cyp3a11 mRNA were expressed constitutively in mouse hepatocytes. Induction of the CAR and PXR target genes, Cyp2b10 and Cyp3a11 respectively, was observed with PB in cultured mouse hepatocytes.

Similarly, treatment with low doses of cyproconazole (up to 1μM) resulted in increases in CAR-mediated Cyp2b10 mRNA of up to 2.7-fold relative to the control. Exposure of hepatocytes to cyproconazole at 25 μM elevated mRNA levels of PXR-dependant Cyp3a11 by up to 1.8-fold, compared with the control group. Higher levels than 25 μM significamtly decreased levels of the CAR and PXR target genes, Cyp2b10 and Cyp3a11 respectively.

Cell proliferation as measured by replicative DNA synthesis was increased by both cyproconazole (3.9-fold versus control) and PB (2.0-fold versus control). This response, while consistent with previous published results in vivo, in which mice showed increases in hepatocyte proliferation after cyproconazole or PB administration in the diet. However, this does not explain why the highest level of replicative DNA synthesis coincides with the lowest levels of Cyp2b10 and Cyp3a11 mRNA induction.

4.10.3.9   *In Vitro* Human Hepatocyte Cell Culture

Study 9:   Elcomb, B., (2011b). Cyproconazole – Cytochrome P450 2B, 3A and DNA-Synthesis Induction in Cultured Male Human Hepatocytes. New in vitro human hepatocyte assay.

This study investigated the ability of cyproconazole to induce cytochrome P450 2B (Cyp2B) transcript levels, Cyp3A transcript levels and potential changes in cell proliferation (measured as the 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 known positive controls for induction of Cyp2B/3A transcript levels and cell proliferation, respectively.

Hepatocytes were exposed to cyproconazole at 6 concentrations (0.2, 1, 5, 25, 125 and 500μM) for 96 hours. PB was used at 3 concentrations (10, 100 and 1000 μM), for 96 hours. Vehicle was 0.5% (v/v) dimethyl sulfoxide, also for 96 hours. Cell toxicity/viability was quantified based on the amount of cellular adenosine-5'-triphosphate (ATP), low amounts relative to vehicle controls indicate low viability or increased cytotoxicity.

The key points that arise from this investigation are:

(1.) Treatment with 125 and 500μM cyproconazole resulted in significant cytotoxicity, with intracellular ATP levels being reduced to 67% and 1% of control respectively. There was no measurable RNA isolated from hepatocytes treated with 500 μM cyproconazole.

(2.) There was no cytotoxicity associated with PB.

(3.) Marked dose dependent increases in the expression of CYP2B6 mRNA were observed following treatment of human hepatocytes with PB. A 1.1 - to 5.2-fold induction of CYP2B6 mRNA was observed.

(4.) Cyproconazole induced CYP2B6 mRNA expression in a dose-dependent manner. Cyp2B6 mRNA expression levels were increased from 1.1 to 4.0-fold relative to that in vehicle control cells.

(5.) Marked dose dependent increases (1.4- to 10.1-fold induction) in the expression of CYP3A4 mRNA were observed following treatment of hepatocytes with PB.

(6.) Cyproconazole induced CYP3A4 mRNA expression in a dose-dependent manner up to 25μM, resulting in a 1.7- to 5.6-fold induction of CYP3A4 mRNA.

(7.) No statistically significant changes in replicative DNA synthesis were observed following treatment with either cyproconazole or PB at any concentration tested.

(8.) Treatment with EGF resulted in a statistically significant increase in replicative DNA synthesis of 12-fold versus concurrent control levels.

Treatment of human hepatocytes with either phenobarbital or cyproconazole resulted in the induction of both CYP2B6 and CYP3A4 mRNA transcript levels *without affecting cell proliferation*. In contrast, treatment with EGF induced a marked increase in cell proliferation.

Conclusion: When administered to rats and mice, compounds such as phenobarbital elicit hepatomegaly that is characterised by increased replicative DNA synthesis, cell proliferation, smooth endoplastic reticulum (SER) proliferation and enzyme induction, especially the induction of Cyp2b via the activated nuclear receptor CAR (or Cyp3a via the activated nuclear receptor PXR). Cultured human liver cells have been shown to exhibit CYP2B induction in response to phenobarbital administration, but it is refractory to the cell proliferation response. This often forms the basis of arguments used to question the relevancy for humans of carcinogenicity and proliferative effects observed in studies where rodents are exposed to xenobiotics.

Based on lowered intracellular ATP concentrations, the 500 μM and 125 μM cyproconazole doses are considered to be cytotoxic to human hepatocytes. As a result of significant cytotoxicity, replicative DNA synthesis measurements were not obtained for these two treatment groups.

CYP2B6 and CYP3A4 mRNA were expressed constitutively in human hepatocytes. Strong induction of the CAR- and PXR-target genes, CYP2B6 and CYP3A4 respectively, was observed with PB in cultured human hepatocytes. There were dose-dependent increases of up to 5.2-fold for CYP2B6 and 10.1-fold for CYP3A4.

Cyproconazole significantly induced CAR-mediated CYP2B6 mRNA (up to 4-fold relative to the control) in human hepatocytes. Exposure of human hepatocytes to cyproconazole also elevated mRNA levels of PXR-dependant CYP3A4 by up to 5.6-fold, compared with the control group. Higher levels than 25 μM, i.e. the 125 μM dose group, decreased the induction of CYP3A4 to about 2.4-fold. This lower value of 2.4-fold induction for CYP3A4 mRNA levels at 125 μM cyproconazole may possibly reflect the increased cytotoxicity (ATP levels being 67% of control) at this concentration.

In cultured male human hepatocytes, cell proliferation as measured by replicative DNA synthesis was not observed in response to either treatment with cyproconazole or PB. Treatment with EGF resulted in a statistically significant increase in replicative DNA synthesis of 12-fold versus concurrent control levels. The absence of any increase in replicative DNA synthesis is consistent with species differences in CAR and PXR receptors between humans and rodents.

4.10.3.10 Human Relevance Framework Assessment of Cyproconazole Liver Tumour Induction in Mice.

Cowie, D. E., (2011). Human Relevance Framework Assessment of Cyproconazole Liver Tumor Induction in Mice.

CLH Dossier Submitter's Comment: This report along with an evaluation of the two *in vitro* hepatocyte assays is submitted in its original form as received from the Cyroconazole notifier as an attachment to section 13 of the CLH dossier. It is provided in the interest of transparency. The dossier submitter considers this document to be supplemental. It contains an overview of the notifier's evaluation of the relevancy of cyproconazole to human health in the context of mouse carcinogenicity. The dossier submitter does not refute the involvement of CAR/PXR in liver upon treatment with cyproconazole but does question whether this mechanism is the primary motivator of carcinogenesis in the mouse 18 month study. There is evidence to suggest hepatocyte cytotoxicity with high concentrations of cyproconazole is a confounding factor and may be the primary event leading to carcinogenicity in this case.

Original notifier's conclusions: "Based on the available data, the MOA for liver tumor formation in male mice treated with high doses of CCZ has been established. This MOA involves key events that include an initial activation of CAR, altered CAR-dependent gene transcription, CYP2B isoform induction plus other metabolizing enzyme changes, and a critical key event of increased cell proliferation. Suppression of apoptosis and perturbed liver biochemistry that causes a low plasma cholesterol level and fat vacuolation are also key events at the carcinogenic doses. Comparative studies in hepatocytes have demonstrated a species difference between mouse and human for the pivotal key event of increased cell proliferation; CCZ did not produce cell proliferation in human hepatocytes. Based on this species difference in response, CCZ is unlikely to cause cell proliferation in humans in vivo, and it is therefore unlikely to cause tumors in humans (Table 8, Figure 3). This conclusion is supported further by epidemiology studies that show a lack of tumor response in patients treated with PB, which shares the same MOA as CCZ. In summary, the data support a conclusion that CCZ does not pose a hepatocarcinogenic hazard to humans."

Overall summary of mechanistic studies relating to mouse liver tumour induction.

To further investigate the mode of action for liver oncogenicity in mice, the proliferative behaviour of rat and mouse liver cells as well as the inducibility of phase I and II xenobiotic-metabolising liver enzymes upon short term treatment were assessed. Generally, the pathological responses as observed in the liver cell proliferation study were similar in rats and mice with centrilobular hepatocyte hypertrophy being the major change in both species. Hepatocyte vacuolation was found in both rats and mice but the distribution showed differences between the species. The overall picture obtained with cyproconazole very much resembles that observed with phenobarbital.

Liver cell proliferation after treatment with cyproconazole showed distinct species differences. In the mouse study there was an increase in cell proliferation beginning at day two of treatment. This early increase in cell proliferation most likely represented a rapid response to a stimulus. Subsequently, the liver appeared able to adapt (probably due to metabolic adaptation, such as microsomal activity - as reflected by hepatocyte hypertrophy), and the cell proliferation index decreased to control level. This pattern is comparable to that known for phenobarbital. In the rat study there was no consistent evidence for an increase in cell proliferation during the comparable period. Thus, the species difference observed may reflect the species difference in the oncogenic potential of cyproconazole.

Cyproconazole was found to be a strong phenobarbital-type inducer of xenobiotic-metabolising enzymes in rat and mouse liver (studies 3-4). In the mouse this comprises an induction of cytochrome P450 isoenzymes of subfamily CYP2A, CYP2B and CYP3A and the phase II enzymes microsomal epoxide hydrolase, microsomal UDP-glucuronosyltransferase and cytosolic glutathione S-transferase. Likewise, a mode of action of cyproconazole as a polycyclic aromatic hydrocarbon- or peroxisome type inducer can be excluded as cytochrome P450 isoenzymes of subfamily CYP1A and CYP4A were not or only slightly induced.

The two *in vitro* hepatocyte culture studies illustrate a few important points:

1. Cyproconazole is cytotoxic to liver cells at high concentrations,

2. Both CYP2B and CYP3A mRNA are induced in both mouse and human cells indicating the involvement of both CAR and PXR in the liver's response to exposure to cyproconazole,

3. Both CYP2B and CYP3A mRNA are induced in both mouse and human cells indicating the involvement of both CAR and PXR in the liver's response to exposure to phenobarbital,

4. There was no cytotoxicity associated with phenobarbital,

5. Treatment with both cyproconazole and phenobarbital increases replicative DNA synthesis in mouse liver cells,

6. However, treatment with both cyproconazole and phenobarbital does not increase replicative DNA synthesis in human liver cells, illustrating that human cells may be refractory to the mitogenic effects observed with activated CAR in mice.

The submitted "Human Relevance Framework Assessment of Cyproconazole Liver Tumor Induction in Mice" is thorough and summarises many points. It contains an overview of the original dossier submitter's evaluation of the relevancy of cyproconazole to human health in the context of mouse carcinogenicity. The MSCA dossier submitter does not refute the involvement of CAR/PXR in liver upon treatment with cyproconazole but does question whether this mechanism is the primary motivator of carcinogenesis in the mouse 18 month study.

It may be concluded that there was clear evidence in the mouse for involvement of CAR activation, altered expression of genes involved in cell cycle, hepatocyte proliferation, suppression of apoptosis, liver growth, and single cell necrosis. However, cyproconazole is also cytotoxic to the liver, resulting in degenerative lesions (necrosis, vacuolation) and subsequent liver cell proliferation. These conditions may create an environment where spontaneously mutated liver cells have a proliferative advantage, leading to clonal expansion and the development of pre-neoplastic foci after long-term treatment to form tumours. It was shown clearly in these additional studies that PB and cyproconazole share a common initial event which is CAR receptor activation, and that the expression of certain genes is similar, but a causal link to liver tumourigenesis is not proven in these studies though they do support the notion that cyproconazole may act in a similar manner to PB.

Industry contends that the cyproconazole data are not consistent with a cytotoxic mode of action as persistent regenerative growth and sustained proliferation are not observed. The effects with cyproconazole are transient, compared to sustained effects with true cytotoxic MoA such as carbon tetrachloride and chloroform. Further details are available in the Human relevance framework submitted by Cowie (2011).

There is little doubt that CAR and PXR involvement are important factors in the hepatic carcinogenesis observed in the mouse long-term dietary study but this is not the only factor involved. Other events downstream of this system or CAR-independent events influenced by cyproconazole and PB exposure may also be operating. Significant hepatocyte cytotoxicity is apparent from cyproconazole treatment alone – the mitogenic effects of nuclear receptors that act as transcription factors serve to further promote the development of liver cancer. The cytotoxicity of cyproconazole and its role in the development of liver tumours cannot be ruled out. In this case it is not sufficient to base a hypothesis of no relevance to man by only taking into consideration the species differences in CAR/PXR downstream events which increase replicative DNA synthesis in one species but not the other.

4.10.4   Summary and discussion of carcinogenicity

There were no treatment-related neoplasms observed in male or female rats even at the highest dose level of 350 ppm (equivalent to 15.59 and 21.76 mg/kg/day in males and females respectively) in the two year combined chronic toxicity and carcinogenicity study. Thus cyproconazole was not considered to be carcinogenic in rats.

Cyproconazole was carcinogenic in mice (Table 34-8). Long-term administration of cyproconazole at doses of 100 ppm (equivalent to 13.17 mg/kg bw/day) and above caused a significant increase in the incidence of hepatocytic adenomas and carcinomas in males. In females these neoplastic changes were observed at doses of 200 ppm (equivalent to 36.30 mg/kg/day).

There appears to be a clear species difference in the oncogenic potential of cyproconazole. Supplementary investigative studies (see studies 1-4 above) suggest a cytotoxic mode of action in mice by which continuous treatment with cyproconazole leads to a well-defined sequence of events, starting with a perturbation of hepatic homeostasis and resulting in degenerative lesions with subsequent liver cell proliferation leading to preneoplastic lesions and finally hepatocellular tumours. Although several of these events were also observed in rats treated with cyproconazole, the incidence of liver tumours did not increase In addition (studies 5-7), investigation into the possible activation of CAR leading to downstream cellular proliferation and tumourigenesis using the CAR null C^{3}H mouse, provided evidence for CAR activation (in a manner similar to phenobarbitol). It was proposed by the notifier that the mechanistic evidence provided suggests that

the mechanism of tumour induction (through activation of CAR and downstream tumourigenesis similar to phenobarbitol), as observed in the mouse, was not relevant to man.

Phenobarbital is a prototypical activator of rodent CAR/PXR, although it does not exhibit direct binding to the ligand binding domain of the CAR receptor itself (Kakizaki et al., 2003). Phenobarbital and similar acting compounds (i.e. CAR activators) had long been observed to induce microsomal enzyme systems. Short term administration of phenobarbital to rodents leads to hepatocellular hypertrophy, hyperplasia, and overall hepatomegaly. Chronic exposure to high doses causes hepatocellular adenomas in both mice and rats and hepatocellular carcinomas in some strains of mice (Thorpe and Walker, 1973; Rossi et al., 1977); however, long-term therapy with phenobarbital has not been found to cause human tumours (Whysner et al., 1996). Inter-individual and species differences in the levels of CAR and the existence of splice variants have also been reported, and it was suggested that these features may play a role in the variability of CAR-dependent liver induction responses (Nuclear Receptors Nomenclature Committee, 1999). Phenobarbital induces xenobiotic metabolizing enzymes in both human and rodent hepatocytes, but the molecular basis for species differences in carcinogenic response has yet to be elucidated fully. The well-known differences in rodent and human xenobiotic responses raise the issue of the relevance of these rodent results to liver carcinogenesis in humans. Studies in hCAR mice indicate that CAR activators also increase DNA replication and inhibition of apoptosis (Huang et al 2005). It is unclear if this would result in the promotion of tumourigenesis in humans. Generally, most study authors agree that long-term barbituate treatment (a strong activator of CAR) is not associated with an increased incidence of liver tumours in humans (Olsen *et al* 1995) though there have been isolated reports suggesting otherwise (Ferko *et al*, 2003; Vazquez *et* Marigil 1989). In addition, prolonged administration of PB in human studies has been shown to increase liver size, which is associated with hepatocellular hypertrophy (Aiges 1980).

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4.10.5   Comparison with criteria

The CLP criteria for classification as a category 2 Carcinogen are as follows:

*"Substances are classified as a category 2 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 cyproconazole. The experimental data presented above demonstrate that cyproconazole is not carcinogenic in the rat. However, at doses of 100 ppm (equivalent to 13.17 mg/kg bw/day) and above, cyproconazole caused a significant increase in the incidence of hepatocytic adenomas and carcinomas in male mice. In females these neoplastic changes were observed at doses of 200 ppm (equivalent to 36.30 mg/kg/day).

In the context of the above criteria, tumours are induced in both sexes of the mouse and not induced in the rat. Significant liver effects including evidence of metabolic induction and hepatotoxicity toxicity was seen in all species tested (rat, mouse, dog) *via* the oral, inhalation and possibly dermal route. The dose of 300 ppm (43.8/70.2 mg/kg bw/day) was an MTD in the 90-day mouse study, based on body weight effects and structural liver changes and clear hepatotoxicity was seen in the chronic toxicity/carcinogenicity study also. Although liver toxicity was seen in all species tested, only the mouse developed tumours. Cyproconazole was not genotoxic. The case presented by the applicant (industry) for human non-relevance of the mouse liver tumours observed relies on comparison to the CAR interaction profile of phenobarbitol (as described above). Studies to explore the mechanism and possible human relevence using the CAR null C^3H mouse, indicated that CAR activation occurred leading to downstream cellular proliferation and possibly tumourigenesis (in a manner proposed to be similar to phenobarbitol). Phenobarbital induces xenobiotic metabolizing enzymes in both human and rodent hepatocytes, but the molecular basis for species differences in carcinogenic response has yet to be elucidated fully. The well-known differences in rodent and human xenobiotic responses raise the issue of the relevance of these rodent results to liver carcinogenesis in humans.

Overall, the evidence is considered to be limited, i.e., the data suggest a carcinogenic effect but are limited in the context of making a definitive evaluation because (1.) tumours are induced in mouse liver only; and (2.) tumours occur at doses toxic to the liver. The mechanism is not unequivocally demonstrated and could involve cytotoxicity (relevant to humans) and/or species specific CAR/PXR downstream events (with questionable relevance to man). Classification in Category 2 is proposed.

Conclusions on classification and labelling

Classification in Category 2 is proposed on the basis of a clear treatment-related increase in mouse liver tumours. The evidence is considered to be 'limited' (single species/questionable relevance to humans) as outlined above.

> **CLP: Carc 2; H351**

4.11   Toxicity for reproduction

Table 35:   Summary table of relevant reproductive toxicity studies

Method	Findings	Remarks	Reference
Kfm: WIST, outbred, SPF quality rats/dietary/2 generation, Cyproconazole (95.8% purity). 4, 20, 120 ppm equiv to F0 (m/f): 0.28/0.33, 1.39/1.67, 8.29/9.88 mg/kg bw day. F1(m/f): 0.37/0.45, 1.77/2.16, 10.88/13.30 mg/kg bw/day. OECD 416. <u>Acceptable</u>	**LOAEL: 20 ppm** Increased liver weight, liver fatty change; slightly increased pre-/peri- and post natal losses	Minimal parental toxicity at LOAEL.	Eschbach. B., 1987. SAN619/5984 and Project no. 380-R. DAR Vol 3 B.6.6.1.1
Range-finding developmental toxicity/gavage/Wistar.HAN rats. Cyproconazole (95.6% purity) 0, 7.5, 30, 75 and 120 mg/kg bw/day. <u>Acceptable</u>	**Maternal NOAEL:** 7.5 mg/kg -Reduced maternal body weight gain in early treatment period **Developmental NOAEL**: 7.5 mg/kg --increased postimplantation loss, reduced foetal body weight; malformations (cleft palate)	The two higher dose levels in this range finding study were considered to be excessively toxic.	Becker, 1985a Report No. RCC 048701. DAR Vol 3 B.6.6.2.1
Developmental toxicity/gavage/ Wistar.HAN rats. Cyproconazole (95.6% purity) 0, 6, 12, 24 and 48 mg/kg bw/day. OECD 414. <u>Acceptable</u>	**Maternal LOAEL:** 12 mg/kg - Reduced maternal body weight gain in early treatment period. **Developmental LOAEL**: 12 mg/kg -increased post-implantation loss, reduced foetal body weight; malformations (cleft palate, hydrocephali); retarded ossification.	-	Becker, H., 1985b. Report No RCC 048712. DAR Vol 3 B.6.6.2.2a
Developmental toxicity (gavage). Machera, 1995. Cyproconazole (purity not stated) 20, 50 and 75 mg/kg bw/day. Equivalent to OECD 414. <u>Acceptable, limited but supplementary</u>.	**Maternal LOAEL:** not found Reduced maternal body weight gain in early treatment period at all doses, marked at 75 mg/kg **Dev. LOAEL:** not found -reduced mean foetal weight from 20 mg/kg -increased post-implantation loss, from 50 mg/kg; malformations (cleft palate, hydrocephali) from 20 mg/kg; retarded ossification from 50 mg/kg.	Limited maternal data. 75 mg/kg clearly toxic but effects at <75 mg/kg not clear. No maternal or developmental NOAEL.	Machera, K, 1995. Syngenta File No. SAN619/5156. DAR Vol. 3B.6.6.2.2.b
Developmental tox Study In Chincilla Rabbits .	**Maternal LOAEL:** 10 mg/kg loss of maternal body weight	There was a large deviation in homogeneity from	Becker H (1986). Report No. RCC

Cyproconazole technical, purity 95.6 % 0, 2, 10, and 50 mg/kg bw OECD 414 Supplementary	and reduced food consumption in early treatment period **Dev LOAEL**: 2 mg/kg increased postimplantation loss.	+28.6% to –35.7% of the mean value. Exposure levels uncertain.	053886 DAR Vol. 3 B.6.6.2.3a
Developmental tox Study In NZW Rabbits . Cyproconazole technical, purity 94.8 % 0, 2, 10, and 50 mg/kg bw OECD 414 Acceptable	**Maternal LOAEL:** 10 mg/kg loss of maternal body weight and reduced food consumption in early treatment period; **Dev LOAEL:** 2 mg/kg -increased incidence of foetal malformations.	Not available for assessment leading to current Annex VI classification	Muller, W. (1991) Report No. 252-060. DAR Vol3 B.6.6.2.3b

4.11.1   Effects on fertility

4.11.1.1   Non-human information

Study 1: 2-generation dietary study in KFM-Wistar rats. Eschbach. B., 1987. SAN619/5984 and Project no. 380-R.   DAR Vol 3 B.6.6.1.1

In a fully guideline compliant study, KFM-Wistar rats) received cyproconazole in the diet at concentrations of either 0, 4, 20 or 120 ppm to assess its potential for toxicity to reproduction over two generations. No treatment-related clinical signs were noted in either the F_2 or F_1 parental generation and no parental animals died during treatment. One female of the mid-dose group and one from the F_0 high dose groups were sacrificed because of total litter losses on day 1 and 5 post-partum, respectively. In addition, a single F_0 high dose female, which failed to give birth, was sacrificed on day 25 post-coitum. A single high dose F_1 female was sacrificed following complete postnatal litter loss on day 4 post-partum. Body weight and food consumption were comparable between treated groups and controls during the entire study period. A marginal/slight increase in relative liver weight was seen in F_0 males (5.5% greater than controls) and females (4.4% greater than controls), attaining statistical significance in males only ($2p > 0.05$).

Findings on reproduction

The summary of reproductive and litter findings are summarised below.

Table 36: Summary of relevant parameter findings

Parameter	Generation	0 ppm	4 ppm	20 ppm	120 ppm
Females on study	F0 F1	26 26	26 26	26 26	26 26
Females mated (Mating index (%))	F0 F1	26 (100) 26 (100)	26 (100) 26 (100)	26 (100) 26 (100)	26 (100) 25 (96.2)
Females pregnant (Fertility index (%))	F0 F1	26 (100) 22 (84.6)	23 (88.5) 22 (84.6)	24 (92.3) 24 (92.3)	25 (96.2) 22 (88.0)

Parameter	Generation	0 ppm	4 ppm	20 ppm	120 ppm
Females with liveborn (Gestation index (%))	F0 F1	26 (100) 22 (100)	23 (100) 22 (100)	24 (100) 24 (100)	24[a] (96.0) 22 (100)
Mean duration of gestation (days)	F0 F1	22.3 22.3	22.4 22.5	22.6 ↑ 22.2	22.5 ↑ 22.3
Mean implantation site per dam	F0 F1	12.5 12.4	12.4 12.4	12.4 12.7	11.6 ↓ 12.4

a)   One female failed to deliver and was killed

Indices reflecting mating success were comparable between treated groups and controls. The fertility indices in the F_0 and F_1 females were comparable to controls. Numbers of implantation sites per dam did not significantly differ among groups. The mean pregnancy length in the low dose F_0 and F_1 females was comparable to their respective controls. An increased gestation length was observed in some mid- and high-dose F_0 females. A single mid-dose animal (no. 73) delivered a single pup on day 24 of gestation. A single female of the high F_0 group failed to deliver and was not included in the calculations of gestation length. However, this female had only a single implantation site and no foetuses at termination. In the F_0 generation the females delivered on days 22 or 23, however the distribution between those two days varied among groups. Delivery on day 22 occurred for 66% of the controls and 50% of the dams in the 20 and 120 ppm groups, indicating a slight increase in the length of pregnancy in some animals of these dose groups, which may have been related to treatment. However, there was no such increase in the F_1 generation dams.

Litter data:

There was a slight reduction in mean implantation sites in the F_0 high dose animals (-7% vs controls). A decreased litter size at birth was seen in this group (-12% than controls). Neither parameter was affected in the F_1 generation. Among pups in the F_1 generation there was a dose-related increase in pre/perinatal mortality in the mid- and high-dose groups (13.6% and 16.3%, respectively). The per/perinatal mortality was also considered relatively high in the control group by the author at 10.7%. There was a corresponding slight increase in postnatal mortality (days 0 – 21 p.p) in the mid- and high-dose groups (6.6%c and 8.1%). Post-natal days 0 – 4 were most affected. However, the apparent increases at 20 ppm in pre/peri and post natal mortality in the F1 litters result from one single F_0-dam (no. 73) which lost 100% of its pups (12/13 pre/perinatal losses, the single surviving pup dying in the first 4 days). As there was no effect in the F1 generation, this event was unlikely to be treatment-related. In the F_2 litters there was a slight increase in pre/perinatal mortality in the high dose only. There was a slightly higher post-natal mortality during days 0-4 in the F_2 high dose group only (7.6% greater than controls). Pup weights were not affected in either F_0 or F_1. No treatment-related alterations were identified in pups on postmortum examination

Conclusion

Administration of cyproconazole in the diet of rats in this two-generation reproduction study produced minimal signs of parental toxicity in F_0 males at the highest dose tested. A slight (statistically significant) increase in relative liver weight was associated with a marginally increased incidence in liver fatty change. This observation is of questionable significance as no toxicity was seen in F_0 females or F1 males and females. In addition, the 90-day rat studies indicated that the NOEL for liver effects was in excess of 80 F_0 ppm (6.4 mg/kg bw/day).

An increase in gestation in some high dose females may have been related to treatment, but was not observed in the F_1 generation. In the F_0 generation there was a slight, statistically non-significant

decrease in the number of implants in the high-dose group. In addition, there was a dose-related increase in pre/perinatal mortality in the high-dose groups in the F_0 and F_1 generation (16.3% and 12.6%, respectively). There was a corresponding slight increase in postnatal mortality (days 0 – 21 p.p) in the high-dose group of the F_1 and F_2 (8.1% and 7.6%, respectively). Treatment with cyproconazole had no effects at 4 ppm. The dose level of 20 ppm could be regarded as a NOAEL in this study if the increased gestation length and litter loss of the single 20 ppm female are disregarded. This dose corresponds to 1.4 and 1.7 mg/kg bw in F_0 males and females and 1.8 and 2.16 mg/kg bw/day in F_1 males and females, respectively

4.11.1.2   Human information

No information

4.11.2   Developmental toxicity

4.11.2.1   Non-human information

Study 1: Dose-Finding Developmental toxicity Study In Wistar/HAN rats with cyproconazole. Becker, 1985a. Report No. RCC 048701. DAR Vol 3 B.6.6.2.1

5/dose level mated Wistar/HAN rats were treated from days 6-15 of gestation at dose levels of 0, 7.5, 30, 75 and 120 mg/kg bw by oral gavage in a study primarily designed to select dose levels for the main study.

Findings:

Maternal: There were no maternal deaths and no clinical signs of toxicity. Body weight gain was reduced only in the high dose animals (2/5 with live foetuses) in the early days of treatment. Mean body weights were lower from 30 mg/kg bw/day upwards, but this was due to the greatly reduced number of foetuses. Food consumption was reduced in the 75 and 120 mg/kg bw/day groups throughout treatment amounting to 10% less than controls at 75 mg/kg bw/day and 16.7% less at 120 mg/kg bw/day.

Developmental: Post-implantation loss was increased in a dose- and treatment-related manner from 30 mg/kg bw/day (7.8%, 2.1%, 24.2%, 64% and 82.3% in controls, 7.5, 30, 75 and 120 mg/kg bw/day groups, respectively). 2/5 females at 75 mg/kg bw/day and 3/5 at 120 mg/kg bw/day had total embryonic resorptions.

External examination of foetuses revealed the following abnormalities: (Control: no malformations or anomalies)
7.5 mg/kg   no malformations or anomalies
30 mg/kg   1 palatoschisis (in 47 foetuses)
75 mg/kg   1 palatoschisis (in 16 foetuses)
120 mg/kg   10 of 11 foetuses with palatoschisis (2 litters)

Table 37: Summary of developmental findings

Parameter	Dose (mg/kg)				
	0	**7.5**	**30**	**75**	**120**
Number of females on study	5	4	5	5	5
Number of females with total resorption	0	0	0	2↑	3↑
Number of females with live foetuses at necropsy	5	4	5	3	2
Number of corpora lutea: per group	55	50	63	53	64
mean per dam	11.0	12.5	12.6	10.6	12.8
Number of implantations:					
-per group	51	47	62	50	62
-mean per dam	10.2	11.8	12.4	10.0	12.4
Preimplantation loss:					
-per group	4	3	1	3	2
-% of corpora lutea	7.3	6.0	1.6	5.7	3.1
-mean per dam	0.8	0.8	0.2	0.7	1.0
Live foetuses: per group	47	46	47	16↓	11↓
Dead foetuses:	0	0	0	0	0
Early resorptions:					
-per group	4	1	15↑	32↑↑	51↑↑
-mean per dam	0.8	0.3	3.0↑	6.4↑↑	10.2↑↑
-% of implantations	7.8	2.1	24.2↑	64.0↑↑	82.3↑↑
Late resorptions:	0	0	0	2	0
-per group					
Postimplantation loss					
-per group	4	1	15↑	34↑↑	51↑↑
-% of implantations	7.8	2.1	24.2	68.0	82.3
-Mean per dam	0.8	0.3	3.0	6.8	10.2
Mean weight of live foetuses:	4.7	4.9	4.1	4.0	4.3

no statistical evaluation of data has been performed

Some maternal toxicity in the form of body weight gain reduction during the early treatment period was seen at 75 and 120 mg/kg bw/day. Body weight loss at 30 mg/kg and above was associated with reduction in foetus number. At 75 and 120 mg/kg the body weight effects were associated with reduced food consumption. Severe embryotoxicity was manifested as a dose related increase in early resorptions at 30 mg/kg and above. Single foetuses with palatoschisis (cleft palate) were found in the 30 and 75 mg/kg groups each. In the high dose group 10 of the 11 viable foetuses had a palatoschisis.

The two higher dose levels in this range finding study were considered to be excessively embryotoxic and were therefore considered to be too high for the main teratogenicity study.

Study 2: Teratogenicity Study In Rats With SAN 619. Becker, H., 1985b. Report No RCC 048712. DAR Vol 3 B.6.6.2.2a.
The purpose of this study was to assess the potential of the test substance to induce structural and/or functional abnormalities in the foetus from exposure during the period of organogenesis. 25/group mated female Wistar/HAN rat (Kfm: WIST, outbred, SPF quality) were treated at dose levels of 0, 6, 12, 24 and 48 mg/kg bw by oral gavage from day 6 of gestation until day 15.

Findings:

Maternal: There was a reduction in mean food consumption in the 24 and 48 mg/kg bw/day from days 6 – 11 and days 11 – 16 and in body weights (body weight loss) from day 6-7 and from day 7-8 of treatment in both the 24 and 48 mg/kg bw/day groups. Body weight gain recovered in the following days. There was an overall reduction in body weight gain for the period 6-11. Mean body weight gain was similar to controls for the remaining period at all dose levels. Mean body weights were statistically significant lower (p < 0.05) from day 12 of treatment at ≥ 24 mg/kg bw/day. This was due to the reduction in uterus weight (reduced foetus number) at these dose levels.

Reproductive: Reproduction parameters were not affected in the 6 and 12 mg/kg groups. At 24 and 48 mg/kg a significantly increased post implantation loss consisting mainly of early (embryonic) resorptions, total resorptions and to a lesser extent late resorptions, amounted to 22.2 % and 30.6 % of implantations at 24 and 48 mg/kg, respectively. A single female of the high dose group had resorptions only; inclusion of this female increased the percentage post implantation loss for this group to 34.2%. Pre-implantation loss was not affected by treatment. The mean foetal bodyweights in the 24 and 48 mg/kg groups were significantly reduced (-8.3 %) as compared to that of the control.

Table 38:   Summary of reproductive parameters

Parameter	0 mg/kg	6 mg/kg	12 mg/kg	24 mg/kg	48 mg/kg
Number of females on study	25	25	25	25	25
Number of pregnant females (%)	24 (96)	22 (88)	23 (92)	25 (100)	23 (92)
Number of non-pregnant females	1	3	2	0	2
Number of females with 100 % intrauterine deaths	1	0	0	0	1
Number of females with live foetuses at necropsy	23	22	23	25	22
Number of corpora lutea: per group mean per dam	286 12.4	277 12.6	296 12.9	299 12.0	281 12.8
Number of implantations: per group mean per dam	261 11.3	259 11.8	279 12.1	279 12.1	255 11.6
Preimplantation loss: per group mean per dam	25 1.1	18 0.8	17 0.7	20 0.8	26 1.2

Live foetuses: per group
            mean per dam
            % of implantations
            % males
	248
	10.8
	95.0
	49.2
		248
		11.3
		95.8
		46.8
			271
			11.8
			97.1
			48.3
				217
				8.7*
				77.8
				45.2
					177
					8.0*
					69.4
					48.9

Dead foetuses	0	0	0	2	1

Early resorptions: per group
            mean per dam
            % of implantations
	12
	0.5
	4.6
		10
		0.5
		3.9
			8
			0.3
			2.9
				56
				2.2
				20.1
					65
					3.0
					25.5

Late resorptions: per group
            mean per dam
            % of implantations
	1
	0.0
	0.4
		1
		0.0
		0.4
			0
			0.0
			0.0
				4
				0.2
				1.4
					12
					0.5
					4.7

Parameter	0 mg/kg	6 mg/kg	12 mg/kg	24 mg/kg	48 mg/kg
Total resorptions: per group	13	11	8	60	77
mean per dam	0.6	0.5	0.3	2.4	3.5
% of implantations	5.0	4.2	2.9	21.5*	30.2*
Postimplantation losses: per group	13	11	8	60	78
mean per dam	0.6	0.5	0.3	2.5	3.5
% of implantations	5.0	4.2	2.9	22.2	30.6
Mean weight of live foetuses:	4.8	4.8	4.7	4.4*	4.4*

*) $P \leq 0.05$

Foetal: Embryo/foetal toxicity was evident at 24 and 48 mg/kg from the following observations; decreased total number of live foetuses per dam, increased numbers of early and late resorptions, decreased foetal bodyweight and incomplete ossification of phalangeal nuclei and the absence of ossification in *calcanea*. At the highest dose one foetus was noted with a *hydrocephalus*, one *hydrocephalus internus* was also apparent and two foetuses had a *palatoschisis* (also reported as cleft palate), of which one was a runt. A single incidence of *hydrocephalus internus* was also apparent at the 24 mg/kg bw/day dose level. Given the probably treatment-related incidence in the 48 mg/kg bw/day, relationship to treatment is assumed.

Table 39:   Summary of Foetal findings

Parameter	0 mg/kg	6 mg/kg	12 mg/kg	24 mg/kg	48 mg/kg
External examinations:					
Number of foetuses examined	248	248	271	217	177
No. of malformations (No. litters affected)	0 (0)	1 (1)	1 (1)	1 (1)	3 (3)
Visceral examinations:					
Number of foetuses examined	78	79	91	71	60
No. of malformations (No. litters affected)	0 (0)	0 (0)	0 (0)	1 (1)	3 (3)
Total number of palatoschisis (litter)					2 (2)
Total number of hydrocephalus (litter)				1 (1)	2 (2)
Skeletal examinations:					
Number of foetuses examined	170	169	180	146	117
No. of anomalies (No. litters affected)	5 (4)	4 (4)	10 (7)	14 (8)	6 (6)
Supernumerary ribs (no.foetuses(%))	7 (2.05)	19(5.6)	31(8.6)	59(20)	65(27.8)

Conclusion:
Mean body weight loss was seen in treated dams at the beginning of the treatment period (days 6-8) from 24 mg/kg bw/day. Mean bodyweight gain recovered thereafter. Mean food consumption was reduced in the 24 and 48 mg/kg bw/day from days 6 – 11 and days 11 – 16.

Embryo/foetal toxicity was evident at 24 and 48 mg/kg from the following observations: decreased total number of live foetuses per dam, increased numbers of early and late resorptions, decreased foetal bodyweight and incomplete ossification of phalangeal nuclei and the absence of ossification in *calcanea*. At the highest dose one foetus was noted with a *hydrocephalus* and two foetuses had a *palatoschisis* (also reported as cleft palate), of which one was a runt. A single incidence of *hydrocephalus internus* was also apparent at the 24 mg/kg bw/day dose level. Given the probably treatment-related incidence in the 48 mg/kg bw/day, relationship to treatment is possible.

Study 3: Developmental Toxicity Of Cyproconazole, an inhibitor of fungal Ergosterol Biosynthesis in the rat. Bull. Environ. Contam. Toxicol. 54; 363-369. Machera K. (1995). Syngenta File No. SAN619/5156. DAR B.6.6.2.2b

In this study, 20/group mated female rats were treated at dose levels of 0, 20, 50 and 75 mg/kg bw by oral gavage from day 6 of gestation until day 16. 100 mg/kg bw was given to 10 pregnant animals in a preliminary test. No analytical data on test substance/vehicle mixtures were reported. The study was not GLP, but considered as OECD 414 guideline equivalent, but with limitations, acceptable as a supplementary study only.

Findings:

Maternal: No treatment related deaths or clinical signs were reported for animals of the main study treated at 75 mg/kg and below. A reduction in weight gain was apparent at 20 mg/kg bw/day. There is little difference in the weight gain reduction from 20 mg/kg bw/day to 50 mg/kg bw/day, while the reduction in weight gain at 75 mg/kg bw/day is clearly marked.

Table 40:   Maternal weight gain

Gestation period	0 mg/kg	20 mg/kg	50 mg/kg	75 mg/kg
Days 0 - 6	12	12	13	10
Days 6 - 11	19	12 (-37)*	12 (-37)*	7 (-63)*
Days 11 - 16	43	21 (-51)*	20 (-53)*	16 (-63)*
Days 6 - 16	62	33 (-47)*	32 (-48)*	22 (-64)*
Days 16 - 21	17	42 (+247)*	28 (+165)*	35 (+265)*
Days 6 - 21	79	75 (-5)	59 (-25)	57 (-28)
net weight change	35	39	37	33

*) $P \leq 0.05$

Reproductive: The dose of 100 mg/kg (preliminary test) was severely toxic to the dams. At this dose level, there was total resorption in 7 out of 10 pregnant dams. From the 12 foetuses obtained, 8 were found dead. A dose dependent increase in the numbers of resorptions was observed over the entire dose range. Statistical significance was achieved at 50 mg/kg and above. Foetal length and foetal body weight were reduced in all treated groups. The reduction in mean body weight was statistically significant in all treated groups, and from 50 mg/kg for mean body length.

Table 41:   Summary of reproductive parameters

Parameter	0 mg/kg	20 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Number of females on study	20	20	20	20	20
Number of litters	17	20	20	20	10[1]
Number of litters with foetuses (live + dead)	17	20	20	17	-
Total number of foetuses	162	151	99	94	-
Number of implantations per litter	10.06	9.00	8.50	9.25	9.00
Total number of foetuses per litter	9.64	7.60	5.20*	4.90*	1.60*

Parameter	0 mg/kg	20 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Number of live foetuses per litter	9.53	7.55	4.95*	4.70*	0.4*
Number of dead foetuses per litter	0.06	0.05	0.20	0.20	1.2*
Number of resorptions per litter	0.41	1.40	3.35*	4.25*	7.40*
Foetal length (cm)	3.45	3.34	3.16*	3.16*	2.68*
Foetal body weight (g)	4.34	3.87*	3.43*	3.40*	2.70*

[1] 7/10 litters totally resorbed.
* Statistically significantly different from control (level not given).

Foetal: Visceral examination revealed increased numbers of cleft palate (also reported as *palatoschisis*), internal *hydrocephalus* and ureterohydronephrosis at all three dose levels. Hydrocephalus at the lowest dose was of moderate degree (including lateral ventricles), whereas at the two higher dose levels full-blown *hydrocephali* (lateral and third ventricle) were observed in addition. Skeletal examination indicated a treatment related retardation of ossification at 50 mg/kg and above. The findings included absence of one or several ossification centres of sternum, absence of 13th rib, reduced number of metatarsal ossification centres (no numbers are given in the publication for any of these findings). Further, larger fontanelles and wider cranial sutures were observed in the two higher dose groups.

Table 42:   Summary of foetal parameters

Observations	0 mg/kg	20 mg/kg	50 mg/kg	75 mg/kg
External observations				
Number of foetuses observed/ number of litters	162/17	151/20	99/20	94/17
Anophthalmia (unilateral)			1 (1)#	
Microphthalmia (unilateral)				1 (1)
Micrognathia of mandibula				2 (1)
Kinky tail		1 (1)		1 (1)
Cachectic		4 (2)		
Visceral observations				
Number of foetuses observed/ number of litters	100/17	86/20	54/20	50/17
Cleft palate		2 (2)	11 (5)	9 (4)
Internal hydrocephalus (moderate degree)		5 (3)	4 (3)	10 (9)
Internal hydrocephalus (full blown)			6 (3)	6 (6)
Ureterohydronephrosis	1 (1)	5 (4)	4 (4)	6 (6)
Skeletal observations				
Number of foetuses observed/ number of litters	62/17	65/20	45/20	44/17
Large fontanelles	1 (1)	2 (2)	7 (3)	2 (2)
Very large fontanelles and cranial sutures, delay of ossification			30 (5)	17 (4)

*) statistically significant difference to control (level of significance not indicated in publication)
#) number of affected foetuses (number of affected litters)

Conclusion:
Maternal toxicity was apparent at all investigated dose levels as reduced body weight gain early during treatment. Foetotoxicity in form of increased foetal resorptions and reduced body weight

and size was statistically significant from 50 mg/kg. Foetotoxicity was also evident in the low dose group in the reduced mean foetal weights. A treatment- and dose-related increase in serious developmental effects was seen at all dose and included cleft palate, internal *hydrocephali* and hydronephrosis of the ureter as well as delayed ossification. The effects were apparent at 20 mg/kg, at lower incidence and/or lower degree of severity. No NOEL could be established in this study for either maternal or embryo/foetotoxicity.

Study 4: Teratogenicity Study In Rabbits With SAN 619 F. Becker H (1986). Report No. RCC 053886. DAR B.6.6.2.3.a

16 mated Chinchilla rabbits females (Kfm: CHIN, hybrids, SPF quality were dosed by oral gavage on days 6-18 of gestation at dose levels of 0, 2, 10 and 50 mg/kg bw in this guideline study. The mean concentration of the test substance ranged from 73.3% to 93.3%. There was a large deviation in homogeneity from +28.6% to –35.7% of the mean value. In addition, the values of the three dose levels after 90 minutes were considerably different from those measured immediately after preparation. The actual exposure levels for the study were therefore uncertain.

Findings:
Maternal: No deaths occurred and no clinical signs of toxicity at any dose level. Body weight development of dams of the two lower dose groups did not differ from that of the control group. High dose animals showed a slight mean loss in body weight between the first and second day of treatment. Body weight gain resumed by day 8 of treatment. Mean corrected body weight gain (body weight gain from day 6 to day 28 minus uterus weight) were similar in all groups. Mean food consumption at 50 mg/kg bw/day group was lower (p<0.05) than controls for days 6-11 post-coitum, and was similar to controls thereafter.

Table 43:   Maternal body weight gain over the treatment period

Group (mg/kg)	Days post-coitum								Corrected body weight gain %*
	0-6 gms (%)	6 – 11 gms (%)	11 – 15 gms (%)	15 – 19 gms (%)	6 – 19 gms (%)	19-24 gms (%)	24-28	6-28	
1 (0)	152(+4.7)	45(+1.3)	85(+2.5)	48(+1.4)	178(+1.4)	60(+1.7)	34(+0.9)	272(+8.0)	-4.9
2 (2)	125(+4.1)	55(+1.7)	75(+2.3)	71(+2.1)	201(+6.3)	68(+20)	40(+1.2)	309(+9.6)	-4.5
3 (10)	160(+5.2)	71(+2.2)	69(+2.1)	59(+1.7)	199(+6.2)	63(+1.8)	37(+1.1)	299(+9.2)	-3.7
4 (50)	187(+6.2)	-12(-0.4)	50(+1.6)	73(+2.2)	111(+3.4)	66(+2.0)	33(+1.0)	210(+6.5)	-4.9

*Corrected body weight gain as a percentage of weight on day 6.

Reproductive: Post-implantation loss was increased from 10 mg/kg bw, statistically significant at 50 mg/kg. The increased implantation losses at 10 mg/kg bw/day were considered treatment-related. The total losses consisted of both early and late resorptions, amounting to 7 % and 16.4 % of implantations at 10 and 50 mg/kg, respectively. The mean foetal bodyweights were not affected.

Table 44:   Summary of relevant reproductive parameters

Parameter	0 mg/kg	2 mg/kg	10 mg/kg	50 mg/kg
Number of mated females	16	16	16	16
Number of non-pregnant females	1	1		
Number of females aborted		1		

Parameter		0 mg/kg	2 mg/kg	10 mg/kg	50 mg/kg
Number of females with live foetuses at necropsy (=numbers used for calculations)		15	14	16	16
Number of corpora lutea:	per group	133	124	137	138
	mean per dam	8.9	8.9	8.6	8.6
Number of implantations:	per group	126	119	129	128
	mean per dam	8.4	8.5	8.1	8.0
Preimplantation loss:	per group	7	5	8	10
	mean per dam	0.5	0.4	0.5	0.6
Live foetuses:	per group	123	118	120	107
	mean per dam	8.2	8.4	7.5	6.7
	% of implantations	97.6	99.2	93.0	83.6
	% males	53.7	46.6	50.8	48.6
Dead foetuses		0	0	0	0
Early resorptions:	per group	3	0	7↑	10
	mean per dam	0.2	0.0	0.4	0.6
	% of implantations	2.4	0.0	5.4	7.8
Late resorptions:	per group	0	1	2	11↑*
	mean per dam	0.0	0.1	0.1	0.7↑
	% of implantations	0.0	0.8	1.6	8.6↑
Total resorptions: (total postimplantation losses)	per group	3	1	9↑	21↑*
	mean per dam	0.2	0.1	0.6↑	1.3↑
	% of implantations	2.4	0.8	7.0↑	16.4↑
Mean weight of live foetuses:		35.1	35.4	36.8	34.2

*) $P \leq 0.05$

Foetal: The following abnormalities were found at external examination:
1 thoracogastroschisis together with arthrogryposis (left forepaw) (control group),
1 omphalocele (10 mg/kg group), and
1 shortened tail (50 mg/kg).

The following abnormalities were found at visceral examination:
1 agenesia of the diaphragm (control),
1 partial agenesia of the diaphragm (10 mg/kg),
1 agenesia of the left kidney and ureter (50 mg/kg),
1 internal hydrocephalus* was noted in each of the treated groups, and
4 foetuses from one litter (10 mg/kg group) were found with slight microphthalmia.

*This is a rare malformation in rabbits according to the historical data submitted (7/3202 controls, 3/7733 inactive treated animals).
A few skeletal anomalies were found, evenly distributed among the groups. None of these findings was considered to be treatment related. There were no apparently treatment-related differences in stage of development (number and/or stage of ossification centres).

Conclusion:
There was a treatment-related decrease in weight gain and food consumption in the high dose group from days 6-11 post-coitum. An apparently treatment and dose-related increase in implantation loss was also seen at 10 mg/kg bw/day, statistically significant at 50 mg/kg. It is noted that the dose levels are considered unreliable.

Study 5: *SAN 619 F - Oral (Gavage) Teratogenicity Study In The Rabbit SAN 619 F - Oral (Gavage) Teratogenicity Study In The Rabbit. Muller, W. (1991). Report No. 252-060. DAR B.6.6.2.3b.*

18 mated NZW rabbits females per group were treated at dose levels of 0, 2, 10 and 50 mg/kg bw by oral gavage, from day 6 to day 18 of gestation in a guideline study. Concentration, homogeneity and stability were considered to be acceptable.

Findings: In the high dose group (50 mg/kg), 15 foetuses from seven litters were malformed. These malformations were found in sternebrae and ribs, vertebral column, hindlimbs, tail and kidneys. In one of these foetuses, a general oedema was also found. When compared with the concurrent and historical control data, a treatment-related effect is concluded for the high dose group. A significant incidence of malrotated hindlimbs was recorded in the high dose animals. There was one incidence of this malformation in the 10 mg/kg bw/day group also. This malformation was not seen in the historical control data presented and association with treatment cannot be excluded at present.

In conclusion, administration of cyproconazole to pregnant rabbits at the dose level of 50 mg/kg bw resulted in body weight loss and reduced food consumption early during the treatment period. Reproduction parameters and mean foetal body weights were not affected at any dose level. An increased incidence of foetal malformations was obtained at the highest dose of 50 mg/kg bw. In addition, the increased incidence of skeletal malformations at 10 mg/kg bw/day may be treatment-related. The occurrence of a single incidence of malrotated hind limbs was noted at 10 mg/kg bw/day. The NOAEL for maternal toxicity is considered to be 10 mg/kg bw/day and 2 mg/kg bw/day for developmental effects.

4.11.2.2   Human information

None available.

4.11.3   Other relevant information

4.11.4   Summary and discussion of reproductive toxicity

Developmental toxicity: Rat
A preliminary range-finding study was carried out in the rat. Maternal toxicity in the form of body weight gain reduction during the early treatment period was seen from ≥75 mg/kg bw/day. Reduced food consumption was associated with this effect. Mean body weight loss observed at 30 mg/kg and above was associated with reduction in foetus number. Severe embryotoxicity was manifested as a dose related increase in early resorptions at 30 mg/kg and above. Single foetuses with palatoschisis (cleft palate) were found in the 30 and 75 mg/kg groups each. In the high dose (120 mg/kg bw/day) group, 10 of the 11 viable foetuses had a palatoschisis. The NOAEL for maternal toxicity was 7.5 mg/kg bw/day and 7.5 mg/kg bw/day for development.

In the main rat developmental toxicity study (0, 6, 12, 24, 48 mg/kg bw), maternal toxicity was apparent at the beginning of the treatment period (days 6-8) from 24 mg/kg bw/day, in the form of mean body weight loss. Mean food consumption was also reduced in this period. Embryo/foetal toxicity was evident at 24 and 48 mg/kg from the following observations: decreased total number of live foetuses per dam, increased numbers of early and late resorptions, decreased foetal bodyweight and incomplete ossification of phalangeal nuclei and the absence of ossification in calcanea. There

were a number of significant malformations at the highest dose (hydrocephalus and palatoschisis). A single incidence of hydrocephalus internus was also apparent at the 24 mg/kg bw/day dose level. The maternal NOEL in this study was 12 mg/kg based on bodyweight gain reduction during early treatment at 24 mg/kg. The developmental NOEL was 12 mg/kg based on reduced foetal bodyweight, increased post implantation loss and increased incidence foetal malformations at 24 and 48 mg/kg.

An independently conducted study (Machera, 1996) was submitted (0, 20, 50, 75 mg/kg bw). In this study, treatment of pregnant rats caused maternal toxicity at all investigated dose levels as manifested by reduced body weight gain early during treatment. The full biological and toxicological relevance of this effect cannot be assessed due to the lack of individual data on weight gains. Foetotoxicity in the form of increased foetal resorption and reduced body weight and size was statistically significant at 50 mg/kg and above. Foetotoxicity was also evident in the low dose group (20 mg/kg bw) in the reduced mean foetal weights. A treatment- and dose-related increase in serious developmental effects was seen at all doses and included cleft palate, internal hydrocephali and hydronephrosis of the ureter as well as delayed ossification. No NOEL could be established in this study, neither for maternal nor for embryo-foetotoxicity.

Developmental toxicity: Rabbit
In the first rabbit developmental study maternal toxicity was apparent at the high dose (50 mg/kg) as a treatment-related decrease in weight gain from days 6-11 post-coitum. A significantly increased post implantation loss was recorded at this dose. An apparently treatment and dose-related increase in implantation loss was also seen at 10 mg/kg bw/day. The maternal NOAEL was considered to be 10 mg/kg bw/day and the developmental NOAEL was 2 mg/kg bw/day.

In a second rabbit study, administration of cyproconazole at the dose level of 50 mg/kg bw resulted in body weight loss and reduced food consumption early during the treatment period. Reproduction parameters and mean foetal body weights were not affected at any dose level. An increased incidence of foetal malformations was obtained at the highest dose of 50 mg/kg bw. In addition, the increased incidence of skeletal malformations at 10 mg/kg bw/day (malrotated hind limbs) may be treatment-related as this finding was considered treatment-related at the high dose. The NOAEL for maternal toxicity was 10 mg/kg bw/day and for developmental effects was 2 mg/kg bw/day.

Comparison with criteria

Cyproconazole did not affect fertility in the rat in a 2-generation study.

The criteria for classification as Cat 1B (H360D May damage the unborn child) are considered to have been met, i.e.

 '....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'.

It was found to cause embryo/foetal toxicity and to induce serious malformations in the two species (rat and rabbit) studied. Developmental toxicity and teratogenicity were found at doses also causing some maternal toxicity which was not considered marked in the context of the criteria.

4.11.5   Conclusions on classification and labelling

Cyproconazole is currently classified as Cat 2 (H361) and was included in Annex 6 of the CLP Regulation. The substance was first discussed in 1994 and the final conclusion was reached in Nov.

1997. The second rabbit study (Muller, 1991 SAN 619F-Oral (Gavage) Teratogenicity Study in the rabbit, No. 252-060, Syngenta SAN619/5393) was not part of the data on which this classification was agreed. On revision of the whole data base in the light of comments made by several member states and EFSA during the original 91/414 peer review and including also consideration of classification criteria of the CLP Regulation (EC 1272/2008 and the Guidance to Regulation (EC) No. 1272/2008 on Classification, Labelling and Packaging of substances and mixtures), a reproductive toxicity classification of Cat 1B; H360d is now proposed by the RMS.

CLP: Repr. 1B; H360d

4.12   Other effects

4.12.1   Non-human information

4.12.1.1   Neurotoxicity

Study: Acute oral Neurotoxicity study in rats. Rached, 2013. Report No. D52431. DAR Vol 3 B.6.7 Addendum 2013

Based on a requirement from the US EPA, Syngenta conducted an acute neurotoxicity study with cyproconazole technical in 2013. Wistar/HAN rats (10 rats/sex/dose) were administered a single oral dose of cyproconazole at dose levels of 0 (control), 10, 50, or 250 mg/kg body weight, and observed for 15 days. A functional observational battery (FOB) was performed once during acclimatisation, on day 1 (at approximately 4 to 6 hours after dosing), and on days 8 and 15. At study termination, animals (5 rats/sex/dose) were perfusion-fixed *in situ*, the brain weights measured and selected nervous system tissues were collected. Histopathological examination was performed on the nervous tissues collected from perfusion-fixed control and high dose rats.

Findings:

General: All animals survived until the end of the study. There were no clinical signs observed for animals treated up to 50 mg/kg. Most high dose animals (250 mg/kg) showed visible weight loss within the first few days. Slight clinical signs were evident in 2 animals prior to day 7, none thereafter.

Functional observational battery: There were no clinical signs observed for animals of either sex treated up to 50 mg/kg. Males on the high dose were unaffected. There were 3 females in the high dose effected (showing signs of reduced activity, decreased rearing, or abnormal gait) and they recovered within the first week of the FOB. Mean body temperature was decreased relative to the control group on only day 1 for all high dose animals. Limb grip strength and landing foot splay were unaffected in all dose groups. Increased locomotor activity was noted on day 1 only for males and females treated in the 50 and 250 mg/kg dose groups.

Organ weights: No effects on absolute, relative and adjusted brain weights were evident.

Macroscopic findings: No gross lesions at necropsy were evident.

Microscopic findings: There were no neurohistopathological lesions due to treatment at any dose level.

Summary and discussion

All cyproconazole-related in-life findings occurred within day 1 post dosing and were transient in nature. All animals recovered during the 2-week post-dose observation period, with the exception of mean overall (day 1-15) body weight gain, which was slightly lower than control values for the 250 mg/kg males and females at study termination.

In the absence of persistent signs of neurotoxicity and any treatment-related neurohistopathological findings, there is no evidence for significant neurotoxicity following a single oral dose of cyproconazole.

Other studies: There is no data for other endpoints such as immunotoxicity. Other specific investigations with cyproconazole have concentrated on determining the mode of action for the liver tumours observed in mice. These are detailed earlier under sections 4.10.3.1 to 4.10.3.10.

4.12.2   Comparison with criteria

Not applicable. No evidence of for significant neurotoxicity following a single oral dose of cyproconazole.

4.12.3   Conclusions on classification and labelling

Cyproconazole does not need to be classified for other effects.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 45: Summary Degradation studies undertaken for Cyproconazole and 1,2,4-triazole

Method	Results	Remarks	Reference
Hydrolysis			
Hydrolysis of ^{14}C-triazole labelled SAN 619 F under laboratory conditions *EU Commission Directive 94/37/EC and 95/36/EC both amending Council Directive 91/414/EEC: Annex I: 2.9.1 Hydrolysis rate and Annex II: 7.2.1.1 Hydrolytic degradation.* *OECD Guideline for testing chemicals, Hydrolysis as a function of pH, 111, Adopted May 1981.*	Cyproconazole did not hydrolyse during the preliminary test at 50°C at any of the 4 pH values. Due to the hydrolytic stability of the parent compound in all buffer solutions, the hydrolysis rate constants and the DT_{50} and DT_{90} values could not be determined. Cyproconazole is hydrolytically stable at environmentally pH values	Compliant with "Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles," March 1986.	Glänzel A., (1999) Hydrolysis of ^{14}C-triazole labelled SAN 619 F under laboratory conditions. Novartis Crop Protection AG, Basel, Switzerland. Study number 99AG04 (Syngenta N° SAN619/6849), 21 December 1999. CAR IIIA 7.1.1.1.1/01

Determination of the Hydrolysis Rate Constants of 1,2,4-H-Triazole Study was carried out prior to the publication of current methods. However, there are no significant differences between the method employed and that later recommended as: *US Environmental Protection Agency (1982) 40 CFR 158; Pesticide Assessment Guidelines, Subdivision N: Environmental Fate; Series 161-1, Hydrolysis studies.*	1,2,4-triazole did not hydrolyse during the study at 25°C at any of the 3 pH values. Due to the hydrolytic stability of the test item in all buffer solutions, the hydrolysis rate constants and the DT_{50} and DT_{90} values could not be determined. 1,2,4-triazole does not hydrolyse between pH 5 and 9.	Study acceptable. The study was conducted prior to implementation of GLP, but inspected by internal quality assurance.	Spare W.C. (1983) Determination of the Hydrolysis Rate Constants of 1,2,4-H-Triazole. Biospherics Incorporated, 4928 Wyaconda Road, Rockville, Maryland, USA. Project number 83-E-074 (Syngenta N° CGA71019/0033) 20 September 1983 CAR IIIA 7.1.1.1.1/02
Photolysis			
SAN 619F: Aqueous photolysis of ^{14}C-triazole labelled SAN 619F *EU Commission Directive 94/37/EC amending Council Directive 91/414/EEC: Annex I: 2.9.2 Photolysis rate*	From the UV-Visible spectra of cyproconazole in pH 7.02 buffer, no significant absorbance was detected in the region 290 nm to 800 nm. This indicates that no direct photolysis of cyproconazole will occur by sunlight. *Cyproconazole is stable to photolysis under environmentally relevant conditions*	Compliant with United Kingdom Good Laboratory Practice Regulations 1999 (in accord with OECD Principles of Good Laboratory Practice [Revised 1997])	Oliver S, Hurt AD, 2002, SAN 619F: Aqueous photolysis of ^{14}C-triazole labelled SAN 619F Syngenta, Jealott's Hill International Research Centre, UK Laboratory report number RJ3322B (Syngenta N° SAN619/7282), 17 December 2002 CAR IIIA 7.1.1.1.2/01

Hydrolysis

Hydrolysis of cyproconazole and its metabolite 1,2,4-triazole was investigated in sterile buffer aqueous solutions (pH 4, 7, and 9). In these experiments hydrolysis was negligible, and it is not considered to contribute to the environmental degradation of cyproconazole or its metabolite 1,2,4-triazole.

Table 46: Hydrolysis of Cyproconazole and 1,2,4-triazole

pH	Temp. [°C]	Initial TS C_0	DT_{50} (d)	r^2	% remaining after test duration	Ref.
[14]C-triazole labelled cyproconazole						
4 7 9	5 d at 50°C	5 ppm	---	---	~100 %	CAR Doc. III A7.1.1.1.1/01

[14]C 1,2,4-Triazole						
4 7 9	30 d at 25°C	10 ppm	---	---	>90 % at all pHs tested.	CAR Doc. III A7.1.1.1.1/02

The solubility of cyproconazole is 93 mg/L at 22 °C (pH 7.1) (98.9%)

Photolysis in water

The UV-Visible spectrum of cyproconazole exhibited no significant absorbance in the region 290 nm to 800 nm. This indicates that no direct photolysis of cyproconazole will occur by sunlight. Solutions of cyproconazole in methanol gave molar absorption coefficients at 290 nm of 3.37 L mol^{-1} cm^{-1} and 1.62 L mol^{-1} cm^{-1} for the 1,384 mg/L and 3,608 mg/L test solutions respectively. Since the molar absorption coefficients are < 10 L mol^{-1} cm^{-1} at wavelengths ≥ 290 nm, no further studies concerning the direct phototransformation in sunlight are required.

Table 47: Absorption behaviour of cyproconazole in the region 290 nm to 800 nm

pH	Temp. [°C]	Concentration of cyproconazole in methanol (mg/L)	Molar absorption coefficients at 290 nm (L mol^{-1} cm^{-1})	Ref.
7.02	25°C	1,384 3,608	3.37 1.62	CAR Doc. III A7.1.1.1.2/01

The molar absorption coefficient was estimated for the range >290 nm.

No aqueous photolysis study is available. However, cyproconazole is expected to be stable to direct photolysis in water.

5.1.1   Stability

No aqueous photolysis study is available. However, cyproconazole is expected to be stable to direct photolysis in water.

5.1.2   Biodegradation

5.1.2.1 Biodegradation estimation

Table 48:   Summary of Biodegradation studies undertaken for Cyproconazole and metabolites

Method	Results	Remarks	Reference
Biodegradation in sewage treatment plants			
Cyproconazole: Testing of biological degradability with fungal and bacterial cultures	Cyproconazole was added to cultures (*arthrobacter sp, Phanerochaete chrysosporium)* and	Not conducted to an agreed Guideline – Studies initiated after the 25th July 1993 should be performed in accordance	Scholtz R. (1996) Cyproconazole: Testing of biological degradability with fungal

Not conducted to an agreed Guideline	activated sludge and incubated in the dark at 22° C. The addition was made to closed vessels. Each vessel had a vial of 2 M KOH inside to trap any evolved CO_2. Breakdown of the chemical was monitored via evolution of carbon dioxide. The radioactivity in the test cultures and in the KOH solutions was determined by LSC. All three microbial inocula showed normal growth as confirmed by the positive and toxic controls. Low amounts of $^{14}CO_2$ of between 0.5 and 0.7% AR was observed from the activated sludge inocula. No evidence of transformation was observed in either the pure fungal or bacterial cultures.	with GLP. However, this study does not follow this protocol. In addition, the study was not performed according to relevant guidelines such as the OECD 301 series (1992), which screens chemicals for ready biodegradability therefore study deemed unacceptable. This study does not meet the strict criteria laid down in the OECD 301 guidelines and is not regarded as a key study. Cyproconazole is considered not readily biodegradable in the absence of a standard biodegradation study. The European Food Safety Authority (EFSA) reached the same conclusion when this study was evaluated under 91/414/EEC. In the absence of a key study this may be regarded as a formal data gap.	and bacterial cultures. BMG Engineering Ltd., Ifangstr., Zürich-Schilieren, Switzerland. Report number: BMG569-95 (Syngenta N° SAN619/5081), 30 May 1996 CAR IIIA 7.1.1.2.1/01
Water/sediment degradation study			
Anaerobic aquatic metabolism of ^{14}C-cyproconazole *US EPA: Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, § 162-3 Anaerobic Aquatic Metabolism Studies (1982)*	Under anaerobic conditions, ^{14}C-phenyl accounted for 14.7%AR in the water phase and ~82 % AR in the sediment system at the end of the incubation period. ^{14}C-triazolyl accounted for ~18.9 and ~76.1 % AR in soil-water system treated at the end of the incubation period. Dissipation of ^{14}C-Cyproconazole via volatilisation, mineralisation, or bound residue formation was ≤ 0.4 % of AR. No significant degradation of cyproconazole occurs under anaerobic conditions.	Study unacceptable. This study was carried out with a soil-water system, rather than a sediment–water system. This study is deemed unsuitable as an anaerobic aquatic (water-sediment) study. This study has also been submitted and evaluated under Anaerobic degradation	Blumhorst MR. (1995) Anaerobic aquatic metabolism of ^{14}C-cyproconazole EPL Bio-Analytical Services Inc., Harristown, IL, United States Report number: 111S16 (Syngenta N° SAN619/6410) 25 May 1995 CAR IIIA 7.1.2.2.2/01
[U-^{14}C]-Triazole cyproconazole: Route and	An aerobic aquatic dissipation study was	Study acceptable - This is an aerobic water-	Völkel W. (1997) [U-^{14}C]-Triazole

rate of degradation in aerobic aquatic systems *US EPA: Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, § 162-3 Anaerobic Aquatic Metabolism Studies (1982)*	performed in two dark water/sediments at 20°C. Degradation in both systems was very slow (DT$_{50}$ >> 1 year). The main dissipation process from the water phase is partitioning to sediment. Only minor metabolites were found (max. 4.6 % AR after 259 d). Mineralisation was negligible (<1 %AR), and unextractable radioactivity amounted to 3.8 – 10 % AR after 259 days. Cyproconazole reached a maximum of 77.5 % AR in the sediment phase of the river system (day 154) and 81.1 % AR in the sediment phase of the pond system (day 28). At the end of the incubation period, cyproconazole amounted to 88.5 % in the river system (16.1 % water phase, 72.4 % sediment phase) and 79.6 % in the pond system (6.4 % water phase, 73.2 % AR sediment phase). In the sterile test systems, the distribution of radioactivity in the water and sediment phases was similar to that of the non-sterile systems. However, under sterile conditions less parent degraded in the river (91.9 %AR) and pond systems (83.1 %AR). The ratio of the cyproconazole diasteroisomers did not change during the course of the water/sediment study. However, enantiomeric ratios were not checked during these experiments.	sediment study. The guideline cited appears to be incorrect. In addition the actual study report does not reference the US EPA guideline instead it references an EU directive, which is not a guideline. However, the study is generally in accordance with the OECD guideline for aquatic sediment systems. In this study, the data is collected for a period of 259 day instead of the recommended 100 days. The redox potential was observed to shift from 220 mV at the start of the study to 150 –160 mV at the end of the study.	cyproconazole: Route and rate of degradation in aerobic aquatic systems, RCC Umweltchemie AG, Itingen, Switzerland., report number: 61300 Syngenta File No. SAN619/0186, 05.11.1997, not published CAR IIIA 7.1.2.2.2/02
Aerobic degradation in soil			
Cyproconazole (SAN 619F): Laboratory Metabolism Study in a Field Soil	Cyproconazole was degraded with a half-life of 89 days in Flaach soil.	Volatiles were measured in a separate experiment, where significant losses occurred due to	Wisson, M. (1989) Cyproconazole (SAN 619F): Laboratory Metabolism Study in a

Guidelines for the Official Testing of Pesticides of the Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig, Germany, part IV, 4-1, "Verbleib von Pflanzenschutzmitteln im Boden - Abbau, Umwandlung und Metabolismus" (December 1986)		imperfections in the experimental system. The fact that volatiles were not trapped should not impact on the rate of degradation of cyproconazole. DT_{50} values obtained from open systems are considered to be acceptable since incubation conditions were maintained at target temperature and moisture content levels. Study exceeded 120 d by 20 d.	Field Soil, Sandoz Agro Ltd., Basel, Switzerland., report number: 41309 Syngenta File No. SAN619/6064, 24.02.1989 Not published. CAR IIIA 7.2.2.1/01
Cyproconazole: Degradation in Three Types of Soil under Various Conditions (Laboratory Study with Field Soils) *Guidelines for the Application for Approval of a Pesticide, Application concerning the Active Ingredient, 21.1, National Agency of Environmental Protection, Miljostyrelsen, Denmark; Official Testing of Pesticides of the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Braunschweig, Germany, part IV, 4-1, Verbleib von Pflanzenschutzmittlen im Boden - Abbau, Umwandlung und Metabolismus (December 1986)*	Cyproconazole was degraded in laboratory soils with half-lives of 72.4, 132 and 192 days under standard conditions (22°C, 40% MWHC). Under colder or dryer conditions degradation was slower. A lower application rate (0.025 mg a.s./kg) resulted in faster degradation. No degradation occurs in sterile soil.	According to the OECD guideline the study duration should not exceed 120 days. However it may last 6-12 months with additional biomass measurement at end of study. The study was performed for 210 d. Soil biomass is not reported at the end of the study the end of it. Therefore, there is no objective reason to discard this study. Volatiles were not trapped at each sampling time. However, this should not impact on the rate of degradation of cyproconazole. DT_{50} values obtained from open systems are considered to be acceptable since incubation conditions were maintained at target temperature and moisture content levels.	Wisson M. (1990b) Cyproconazole: Degradation in Three Types of Soil under Various Conditions (Laboratory Study with Field Soils), Sandoz Agro Ltd., Basel, Switzerland., report number: 41313 Syngenta File No. SAN619/6143, 24.09.1990 Not published CAR IIIA 7.2.2.1/02
Degradation of 1,2,4-triazole in Three Soils under Aerobic Conditions *SETAC, part 1, 1.1 Aerobic degradation, Ed. M. Lynch (1995) Dutch Board for the Authorisation of Agrochemicals (CTB). G. 1.1.:(1995) U.S. EPA. Pesticide*	1,2,4-triazole is degraded in laboratory soils with a DT_{50} ranging from 6.8 to 12.04 d	Study acceptable	Slangen P.J. (2000) Degradation of 1,2,4-triazole in Three Soils under Aerobic Conditions., NOTOX B.V., 'S Hertogenbosch, Netherlands., report number: NOTOX 278336 Syngenta File No. CGA64250/4345, 26.05.2000

Assessment Guidelines, Subdivion N Chemistry: Environmental fate § 162-1 Aerobic soil metabolism studies (1982) *Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA). Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln im Boden – Abbau, Umwandlung und Metabolismus (1986)*			Not published.
Anaerobic degradation in soil			
Anaerobic aquatic metabolism of ^{14}C-cyproconazole *US EPA: Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, § 162-3 Anaerobic Aquatic Metabolism Studies (1982)*	Under anaerobic conditions, ^{14}C-phenyl accounted for 14.7%AR in the water phase and ~82 % AR in the sediment system at the end of the incubation period. ^{14}C-triazolyl accounted for ~18.9 and ~76.1 % AR in soil-water system treated at the end of the incubation period. Dissipation of ^{14}C-Cyproconazole via volatilisation, mineralisation, or bound residue formation was ≤ 0.4 % of AR. No significant degradation of cyproconazole occurs under anaerobic conditions. Cyproconazole applied to established anaerobic aquatic systems was not degraded.	Study acceptable	Blumhorst M.R. (1995) Anaerobic aquatic metabolism of ^{14}C-cyproconazole, EPL Bio-Analytical Services Inc., Harristown, IL, United States, report number: 111S16 Syngenta File No. SAN619/6410, 25.05.1995 Not published CAR IIIA7.2.2/01
Cyproconazole: Degradation in Soil under Anaerobic Condition (Laboratory Study with a Field Soil) *US EPA: Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, § 162-2 Anaerobic Soil Metabolism Studies (1982)* *National Agency of Environmental Protection, Denmark:*	^{14}C-cyproconazole degraded quickly in aerobic soil, after flooding with water degradation slowed but did not cease until oxygen was depleted from the system. No major metabolites are formed under these conditions.	Acceptable from a rate of degradation point of view. [14]CO$_2$ recoveries are not quantified, and mass balances cannot be calculated. Consequently the study is deficient from a route of degradation point of view.	Wisson M. (1990a) Cyproconazole: Degradation in Soil under Anaerobic Condition (Laboratory Study with a Field Soil), Sandoz Agro Ltd., Basel, Switzerland, report number: 41316Syngenta File No. SAN619/5541, 14.09.1990 Not published CAR IIIA7.2.2/02

Application for Approval of a Pesticide Application concerning the Active Ingredient, 21.1			

5.1.2.2 Screening tests

Not relevant to this dossier.

5.1.2.3 Simulation tests

Not relevant to this dossier.

5.1.3   Summary and discussion of degradation

Cyproconazole is stable to hydrolysis and is expected to be stable to direct photolysis in water (at $\lambda > 290$ nm UV adsorption $\varepsilon < 10$ L-1 mol^{-1} cm^{-1}). Cyproconazole is considered not readily biodegradable in the absence of a validly conducted biodegradation study. Degradation in two dark water/sediments at 20°C was very slow ($DT_{50} \gg 1$ year). The main dissipation process from the water phase is partitioning to sediment. Only minor metabolites were found (max. 4.6 % AR after 259 d). Mineralisation was negligible, and unextractable radioactivity amounted to 3.8 – 10 % AR after 259 days. The ratio of the cyproconazole diastereoisomers did not change during the course of the study. However, enantiomeric ratios were not checked during these experiments.

The route of degradation of cyproconazole in soil under dark aerobic conditions at 20 – 22 ºC was investigated in three studies with ^{14}C triazole-labelled cyproconazole (one soil: pH 7.2), ^{14}C-benzyl-labelled cyproconazole (three soils: pH 4.3 – 7.0), and ^{14}C-phenyl-labelled cyproconazole (one soil: pH 7). Overall, the degradation was investigated in four soils, since one of the soils was investigated with two differently labelled compounds (benzyl and phenyl-labelled cyproconazole). In all these studies degradation of cyproconazole was slow, and considerable amounts of radioactivity remained as unmodified cyproconazole at the end of the respective experiments. One metabolite ≥ 10 % AR was observed (1,2,4-triazole, max. 17.36 % AR after 140 days, end of the study). Only slight variations were observed on the diastereomeric ratios during the experiments. These variations are not significant and consistent enough to consider that diastereomeric degradation occurs. However, enantiomeric ratios were not tested during these experiments.

The degradation of cyproconazole in soil under dark anaerobic conditions was investigated in two studies that show that cyproconazole is stable under anaerobic conditions.

Photolysis of cyproconazole in soil was investigated under simulated sunlight (Xenon lamp filtered for $\lambda < 290$ nm). Cyproconazole was stable in the dark control, and only very slight degradation was observed in the irradiated experiment. It may be considered that photolysis will not contribute to the environmental dissipation of cyproconazole.

Persistence of cyproconazole in soil under dark aerobic conditions was investigated in several studies. Under laboratory conditions, the DT_{50} ranged from 100 d to 427 d at 12°C. The geomean DT_{50} lab calculates to 244d, based on 128.6 days at 20°C. In one study the effect of application rate, temperature, and soil water content was also investigated. Several field dissipation studies were submitted. For some of the field trials the data had to be fitted to DFOP kinetics in order to obtain reliable results. Residues of the major soil metabolite 1,2,4-triazole were not measured in

any of the field studies. In field trials the DT_{50} ranged from 28.97 d (DFOP) to 162 d (SFO). The corresponding DT_{90} ranged from 306.92 d (SFO) to 1,000 days(DFOP).

The rate of degradation of the major soil metabolite 1,2,4-triazole under dark aerobic conditions at 20 ºC was investigated in one study with three soils. Under these conditions the DT_{50} ranged from 6.8-12.04 d.

Photochemical oxidative degradation of cyproconazole and its metabolite 1,2,4-triazole was estimated with the AOPWIN software. The photochemical half-life of cyproconazole in the atmosphere was determined to be around 1 day, and therefore it is not expected to persist in the atmosphere.

5.2   Environmental distribution

5.2.1   Adsorption/Desorption

Cyproconazole exhibits low to medium mobility in soil. The average normalised distribution coefficient for organic carbon content is 473 L/kg. The average K_{foc} is 364 L/kg. The amount of soil adsorbed cyproconazole removed by the 6 desorption steps varied between ~2 and ~60%. Therefore, adsorption was not fully reversible. The soil-water-distribution study on the Cyproconazole metabolite, 1,2,4-triazole (CGA 71019), demonstrated weak adsorption to soil. The adsorption corrected for the organic carbon content of the soils, K_{foc}, has a range of 43 to 120 mL/g, mean 111 mL/g. The desorption equilibrium constants were much higher compared to the adsorption constants and amounted to approximately 1.2 and 4.6 for the first and second desorption cycle, respectively.

Table 49:   Summary of relevant information on aquatic toxicity – Toxicity of Cyproconazole Technical to Aquatic Organisms

Method	Results	Remarks	Reference
Soil-water-distribution of Cyproconazole			
Adsorption, Desorption and Mobility of SAN 619 F in Soil *US EPA Environmental fate § 163-1 Soil adsorption and desorption (1982)* *OECD No. 106*	Cyproconazole exhibits low to medium mobility in soil. No obvious pH dependence is observed from the available data. The average normalised distribution coefficient for organic carbon content is 473 L/kg. The average Kfoc is 364 L/kg. The amount of soil adsorbed cyproconazole removed by the 6 desorption steps varied between ~2 and ~60%. Therefore, adsorption was not fully reversible.	Study performed before GLP was required.	Skinner W. S, Collier KD, Quistad G. B, (1985c) Adsorption, Desorption and Mobility of SAN 619 F in Soil, Zoecon Corp., Palo Alto, United States, Report numbers: 3760-24-11-85 Syngenta File No. SAN619/6102, 11.11.1985, (not published). CAR IIIA 7.2.3.1/01
Soil-water-distribution of metabolites (1,2,4-triazole)			
Soil adsorption and desorption of 1,2,4-Triazole	1,2,4-triazole (CGA 71019) is weakly adsorbed to soil. The adsorption corrected for the organic carbon	Laboratory certified to Good Laboratory Practice regulations, US EPA, 40 CFR Part 160.	Hawkins D.R, (1988) Soil adsorption and desorption of 1,2,4-Triazole, Rohm and Haas Company, 727 Norristown Road, Spring

Environmental fate § 163-1 Soil adsorption and desorption (1982)	content of the soils, Kfoc, has a range of 43 to 120 mL/g, mean 111 mL/g. The results from the Lakeland soil were excluded because of its very low organic carbon content. No obvious pH dependence is observed form the available data. The desorption equilibrium constants were much higher compared to the adsorption constants and amounted to approximately 1.2 and 4.6 for the first and second desorption cycle, respectively.		House, Pennsylvania, USA., Rreport number: 34S-88-27 Syngenta File No. CGA71019/0014, 03.11.1988, (not published). CAR IIIA 7.2.3.1/02

5.2.2   Volatilisation

Not relevant to this dossier.

5.2.3   Distribution modelling

Not relevant to this dossier.

5.3   Aquatic Bioaccumulation

5.3.1   Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Measurements of aquatic and terrestrial bioaccumulation of cyproconazole have been performed. The bioconcentration factor for fish was determined experimentally (to be 28 L/kg.

5.3.1.2 Measured bioaccumulation data

Uptake, depuration and bioconcentration of ^{14}C-SAN 619 F to Bluegill Sunfish (Lepomis macrochirus); Forbis, A.D.(1986):

The uptake, depuration and bioconcentration of ^{14}C-SAN619F was determined in accordance with EPA FIFRA 165-4 Guideline in agreement with OECD 305E.

One hundred and twenty young bluegill sunfish (Lepomis macrochirus) were exposed to a nominal concentration of 0.3 mg cyproconazole/L, for a 28-day flow-through study (absorption phase) followed by a 14-day depuration period (elimination phase). Dimethylformamide (DMF) was used as solvent (vehicle) to prepare the solution (0.1 ml/L). Two additional groups were added as solvent

control and water control. Uptake and depuration of ^{14}C-cyproconazole was determined by radio-analysis in whole body, edible tissue and non-edible portions throughout the study.

Temperature, pH, dissolved oxygen were recorded during the study. Concentration of ^{14}C-cyproconazole in the test solutions was determined by LSC on days 0.17, 1, 3, 7, 14, 21, 28 of the absorption phase. Mortality and behavior were recorded daily until day 42, end of the study. During the study the water temperature ranged from 20 to 23, the dissolved oxygen from 6.5 to 9.2 mg/L and pH from 8.0 to 8.3.

A 7-day preliminary study showed a no observed effect concentration (7-day NOEC) of 5.3 mg/L (mortality, behavior). For the 28 days of the bioconcentration study, no effect on mortality or behavior was observed. The no observed effect concentration over 28 days was at least 0.27 mg/L in bluegill sunfish (28-d NOEC ≥ 0.27 mg/L).

The bioconcentration factor for fish was determined experimentally to be 28 L/kg (Forbis, A.D., 1986, see Doc IIIA, Section A7.4.2/01). With an aquatic bioconcentration factor (BCF) of 28 L/kg, cyproconazole has a low bioaccumulation potential in fish and other aquatic organisms.The octanol/water partition coefficient of the cyproconazole metabolite, CGA 71019 (1,2,4 triazole) (log Pow) is −1, indicating that the compound is unlikely to bioaccumulate in fish or other aquatic organisms. Specific studies on the bioaccumulation of CGA 71019 (1,2,4 triazole) are therefore not considered necessary.

Tissues	Accumulation: absorption phase (Day 28)		Clearance: elimination phase (Day 42)	
	Concentration [mg ai/kg]	Daily BCF	Concentration [mg ai/kg]	Elimination [%]
Edible	1.8 (1.1-4.2)	6.7 (3.9-15)	< 0.3	> 83.3
Whole body	5.9 (2.3-9.1)	22 (8.2-34)	0.41	93
Non-edible	11 (3.6-16)	41 (13-59)	0.61	94

5.3.2   Summary and discussion of aquatic bioaccumulation

Measurements of aquatic and terrestrial bioaccumulation of cyproconazole have been performed. The bioconcentration factor for fish was determined experimentally (Forbis, A.D., 1986, see Doc IIIA, Section A7.4.2/01) to be 28 L/kg.

With an aquatic bioconcentration factor (BCF) of 28 L/kg, cyproconazole has a low bioaccumulation potential in fish and other aquatic organisms.The octanol/water partition coefficient of the cyproconazole metabolite, CGA 71019 (1,2,4 triazole) (log P_{ow}) is −1, indicating that the compound is unlikely to bioaccumulate in fish or other aquatic organisms. Specific studies on the bioaccumulation of CGA 71019 (1,2,4 triazole) are therefore not considered necessary.

5.4   Aquatic toxicity

Table 50:   Summary of relevant information on aquatic toxicity – Toxicity of Cyproconazole Technical to Aquatic Organisms

Method	Results	Remarks	Reference
Acute aquatic toxicity			

Fish: Rainbow trout *Salmo gairdneri* (96 hours). *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988.*	LC_0	LC_50	LC_100		Bowman, J.H. (1988a) Acute toxicity of SAN619F Technical to Rainbow Trout (*Salmo gairdneri*). ABC Analytical Bio-Chemistry Lab. Inc, Columbia Report No. 36546. Syngenta File No. SAN619/5965 (unpublished). 29.01.1988 DAR Vol 3 B9.2.1.1.1
	18 mg a.i./L measured	19 mg a.i./L measured	20 mg a.i./L measured		
Fish: Bluegill Sunfish *Lepomis macrochirus* (96 hours) *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988*	LC_0	LC_50	LC_100		Bowman, J.H. (1988b) Acute Toxicity of SAN 619 F Technical to Bluegill Sunfish (*Lepomis macrochirus*), ABC Analytical Bio-Chemistry Lab. Inc., Columbia, Report number: 36545 Syngenta File No. SAN619/5950 (unpublished) 11.01.1988 DAR Vol 3 B9.2.1.1.2
	18 mg a.i./L measured	21 mg a.i./L measured	24 mg a.i./L measured		
Fish: Carp *Cyprinus carpi* (96 hours) *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988 and OECD 203*	LC_0	LC_50	LC_100		Hamburger, F. and Klotzsche, C. (1985) SAN 619 F - Fish toxicity in the carp, Sandoz AG, Basel, Switzerland, Report number: 149/85. Syngenta File No. SAN619/5961 (not published). 29.07.1985 DAR Vol 3 B9.2.1.1.3
	14.8 mg a.i./L measured	18.9 mg a.i/L measured	29.1 mg a.i./L measured		
Fish: Sheepshead minnow *Cyprinodon variegatus* (96 hours) *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-3, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982.*	LC_0	LC_50	LC_100		Drottar, K.R. *et al.* (1993) Cyproconazole: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*), Wildlife International Ltd. (Easton, MD), Easton, United States, Report number: 131A-151A. Syngenta File No. SAN619/5047
	17 mg a.i./L measured	21 mg a.i./L measured	26 mg a.i./L measured		

					(unpublished). 01.09.1993 DAR Vol 3 B9.2.1.1.4
Invertebrates: *Daphnia magna* (48 hours) *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-2 (October 1982)*	**LC$_0$** 1.5 mg a.i./L measured	**EC$_{50}$** >22 mg a.i./L (highest concentration studied) measured	**LC$_{100}$** >22 mg a.i./L measured		Surprenant D.C. (1986) Acute Toxicity of SAN 619 F To Daphnids (*Daphnia magna*), Springborn Laboratories Inc., Wareham, United States, Report No: BW-86-11-2156. Syngenta File No. SAN619/5937 (unpublished) 13.11.1986 DAR Vol 3 B9.2.4.1.1
Invertebrates: *Daphnia magna* (48 hours) *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-2 (October 1982)*	**LC$_0$** 8.5 mg a.i./L measured	**EC$_{50}$** 26 mg a.i./L measured	**LC$_{100}$** 77mg a.i./L measured		Frazier S. (1988) Acute Toxicity of SAN 619 F to *Daphnia magna*, ABC Analytical Bio-Chemistry Lab. Inc., Columbia, Report No. 36547 . Syngenta File No. SAN619/5938 (unpublished) 16.02.1988 DAR Vol 3 B9.2.4.1.2
Invertebrates: Saltwater mysid (*Mysidopsis bahia*) (new name: *Americamysis bahia*) (96 hours) *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-2 (October 1982) and ASTM Standard E 729-88 (1988)*	**LC$_0$** 2.8 mg a.i./L measured	**LC$_{50}$** 9.6 mg a.i./L measured	**LC$_{100}$** 19 mg a.i./L measured		Drottar K. and Swigert, J.P. (1993b) Cyproconazole: a 96-hour flow-through acute toxicity test with the saltwater mysid (*Mysidopsis bahia*), Wildlife International Ltd. (Easton, MD), Easton, United States, Report No: 131A-150 Syngenta File No. SAN619/5045 (unpublished) 01.09.1993 DAR Vol 3 B9.2.4.1.3
Invertebrates: Eastern oyster (*Crassostrea virginica*) *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-2 (October 1982) and ASTM Standard E 729-88 (1988)*	**LC$_0$** 1.9 mg a.i./L measured	**EC$_{50}$** 2.6 mg a.i./L measured	**LC$_{100}$** 5.4 mg a.i./L measured		Sved D. W. *et al.* (1993) Cyproconazole (SAN 619F): A 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*), Wildlife International Ltd.

							(Easton, MD), Easton, United States, Report No: 131A-149. Syngenta File No. SAN619/5046 (unpublished) 01.09.1993 DAR Vol 3 B9.2.4.1.4
Algae: *Scenedesmus subspicatus* (96 hours) *OECD No.201 (1984)*	$NOEC_b$	E_bC_{50}	E_{r50}	E_{cb100}	72 hr E_bC_{50}		Ellgehausen, H. (1986a) Acute toxicity of SAN 619F to *Secenedesmus subspicatus* (OECD: Algae Growth Inhibition Test). RCC Itigen Report No: 75521 (unpublished). Syngenta Report No. SAN619/0104. 18.11.1986 DAR Vol 3 B9.2.6.1.1
	0.021 mg a.i./L measured	0.077 mg a.i./L measured	0.12 mg a.i./L nominal	5.8 mg a.i./L measured	0.099 mg a.i. /L measured		
Algae: *Chlorella vulgaris* (72 hours) *OECD No.201 (1984)*	$NOEC_b$	E_bC_{50}	E_{r50}	E_{cb100}	72 hr E_bC_{50}		Jenkins, W.R.. (1993) SAN 619F: Determination of EC_{50} to *Chlorella vulgaris* (72-hour static assay). Pharmaco LSR No. 93/SAS049/0830 Syngenta Report No. SAN619/5314 (unpublished). 08.11.1993 DAR Vol 3 B9.2.6.1.2
	0.392mg a.i./L measured	0.66 mg a.i./L measured	1.176mg a.i./L measured	-	0.66 mg a.i./L measured		

Aquatic microbials: Activated Sewage Sludge (respiration inhibition) 3 hours *OECD No. 209*	EC_{20}		EC_{50}		EC_{80}		*Wallace, S.J. (2002) SAN619 (Cyproconazole technical): Effect on the Respiration Rate of Activated Sludge, Brixham Environmental Laboratory, Brixham, United Kingdom, Report No: BL7332/B. Syngenta File No. SAN619/7217 (unpublished) 30.06.2002* DAR Vol 3 B9.10
	-		>100 mg a.i./L nominal		-		

Long-term aquatic toxicity							
Fish: Rainbow trout *Oncorhynchus mykiss* 21 days	LC_0		NOEC				Jenkins, C.A. (1989) SAN 619 F - 21-day rainbow trout toxicity
	0.65 mg/L measured		5.04 mg a.i./L (growth) measured				

OECD No. 204		0.65 mg a.i./L (behavior) measured		study under flow-through exposure conditions, Life Science Research Ltd., Eye, United Kingdom, Report No: 89/SAS032/0197. Syngenta File No. SAN619/5962 (unpublished) 17.07.1989 DAR Vol 3 B9.2.2.1.1.1
Fish: Rainbow trout *Oncorhynchus mykiss* 89 days *U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-4(a) (1982), ASTM Standard E 1241-88 (1988), and U.S. EPA "Standard Evaluation Procedure, Fish Early Life-Stage Test" (1986)*	**LC$_0$**	**NOEC**	The EFSA evaluated cyproconazole as a PPP and concluded that 0.16 mg a.i./L should be the LOEC not the NOEC.	Drottar, K.R and Swigert, J.P. (1993a) Cyproconazole (SAN 619): an early life-stage toxicity test with the Rainbow trout (*Oncorhynchus mykiss*), Wildlife International Ltd. (Easton, MD), Easton, United States, Report number: 131A-153. Syngenta File No. SAN619/5039 (unpublished). 21.12.1993 DAR Vol 3 B9.2.2.2.1
	-	0.58 mg a.i./L (survival) measured 0.16 mg a.i./L LOEC (fry growth) measured NOEC < 0.16 mg a.i./L measured		
Fish: Rainbow trout *Oncorhynchus mykiss* (93 days – 59 days post hatch) OECD Guideline 210 and OPPTS Draft Guideline 850.1400	**LC$_0$**	**NOEC**	Highest concentration tested in this Early Life-Stage test	Wheeler, J.R. (2006) Cyproconazole (SAN619): Early Life-Stage Toxicity to Rainbow Trout (*Oncorhynchus mykiss*), Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK. (Syngenta File No. SAN619/8096) – not published. CAR Doc IIIA A7.4.3.2/03
	-	0.305 mg a.i./L measured		
Fish: Fathead minnow (*Pimephales promelas*) 263 days (90 days post-hatch) Adapted from OPPTS Draft Guideline 850.1500 to include	**LC$_0$**	**NOEC**	Full Fish Life Cycle	Carfarella, M.A. (2009), Cyproconazole - Fish Full Life-Cycle Test with Fathead Minnow (*Pimephales*
	-	0.51 mg a.i./L measured		

endocrine endpoints					*promelas*), Syngenta, Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1037 USA (Syngenta File No. SAN619_10010) – not published. CAR Doc IIIA A7.4.3.2/04
Fish: Fathead minnow *Pimephales promelas* 357 days *Adapted from US-EPA, Fish Life Cycle Toxicity Tests, EPA 540/9-86-137, July 1986, and incorporating biological endpoints proposed by the OECD Endocrine Disrupters Testing and Assessment (EDTA) task force*	**LC$_0$**		**NOEC**		Williams, T.D. (2001) CYPROCONAZOLE tech. (SAN 619): Determination of effects on the life cycle of the fathead minnow (*Pimephales promelas*), including measurements of vitellogenin and gonad histopathology, Brixham Environmental Laboratory, Brixham, United Kingdom,Report No : BL7106/B. Syngenta File No. SAN619/7100 (unpublished) 29.07.2001 DAR Vol 3 B9.2.2.3.1
	-		0.5 mg a.i./L nominal (VTG decrease)- comparison with solvent control nominal		
Invertebrates: *Daphnia magna* 21 days *EPA FIFRA 72-4*	**EC$_{10}$**	**EC$_{50}$**	**NOEC**	Flow-through	Drottar, K.R and Swigert, J.P. (1993c) Cyproconazole (SAN 619 F): a flow-through life-cycle toxicity test with the Cladoceran (*Daphnia magna*), Wildlife International Ltd. (Easton, MD), Easton, United States, Report No: 131A-152. Syngenta File No. SAN619/5043 (unpublished). 07.10.1993 DAR Vol 3 B9.2.5.1.1
	-	-	0.29 mg a.i./L (reproduction) measured >2.3 mg a.i./L (parental generation) measured		
Invertebrates: *Daphnia magna* 21 days OECD Guidelines for Testing of Chemicals, No. 211, *Daphnia magna* Reproduction Test, September 21, 1998	**EC$_{10}$**	**EC$_{50}$**	**NOEC**	Semi-static	Bätscher, R. (2006); cyproconazole (SAN619) technical: effect on survival, growth and reproduction of Daphnia magna in a semi-static test over three weeks. RCC
	-	0.83 mg a.i./L nominal	0.023 mg a.s./L nominal		

		Ltd. Environmental chemistry and farm Pharmanalytics CH-4452 Itingen, Switzerland. Laboratory report number: A99191 (Syngenta File No SAN619/8244), 23.11.2006 – not published. 27.10.2006 CAR Doc IIIA A7.4.3.4/02
Sediment dwelling organisms: *Chironomus riparius (syn. Chironomus thummi* 28 days *OECD Guideline for Testing of Chemicals, Proposal for Toxicity Test with Chironomidae, May 1998*	**NOEC** 5.0 mg a.i./L (via water column) Nominal AND *50 mg a.i./kg (via sediment) dwt sediment emergence and development nominal*	Grade R. (1999) Toxicity test of SAN 619 tech. on sediment-dwelling *Chironomus riparius* (syn. *Chironomus thummi*) under static conditions, Novartis Crop Protection AG, Basel, Switzerland, Report No: 983753. Syngenta File No. SAN619/0627 (unpublished). 07.04.1999 DAR Vol 3 B9.2.7.1

5.4.1   Fish

5.4.1.1 Short-term toxicity to fish

Acute toxicity of SAN619F Technical to Rainbow Trout (Salmo gairdneri); Bowman J.H. January 1988(a):

The acute toxicity of SAN619F technical to *Salmo gairdneri* was investigated by Bowman J.H. in accordance with the *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988*.

In this static fish bioassay, Rainbow Trout (*Oncorhynchus mykiss)*, 10 fish per test concentration, were exposed for 96 hours to nominal concentrations of 1, 10, 18, 32 , 56, 100 and 180 mg SAN619F/L. Dimethylformamide (DMF) was used as the solvent (vehicle) to prepare the test solutions (0.1 ml/L max.). An appropriate solvent control and dilution water control were run in parallel with the test concentrations. The stability of the test solutions was determined at 0 and 96 hours by gas chromatography. Temperature, pH and dissolved oxygen were also recorded at 0 hrs, 48 hrs and 96 hrs. Fish were observed for mortality and sub-lethal effects after 24, 48, 72, and 96 hours exposure and dead fish were removed and any observations recorded. The fish were not fed for the duration of the study and the test vessels were not aerated.

The mean measured test concentrations were 98, 96, 100, 50, 17, 18 and 11% of nominal concentrations (0.98, 9.6, 18, 16, 9.4, 18 and 20 mg/L of SAN 619F, respectively), therefore effects were based on mean measured concentrations. The pH values of the test waters ranged from 6.8 to 7.5. The dissolved oxygen concentrations ranged from 3.5 to 9.3 mg/L during the test, representing 35 and 90% saturation at 13 and 12°C respectively, thus, after 96 hours of exposure, dissolved oxygen fell down below the saturation level recommended by the guidelines in the 10 mg/L and higher test systems. The control chambers remained above 78% saturation throughout the 96-hour study period. These conditions might have had an influence on mortality. A precipitate was also observed during the study in the 10 mg/L and higher test systems, which increased with concentration, indicating that the water solubility was likely to have been exceeded. Conductivity of the well water used by the performing laboratory ranged from 500 – 650 μmhos/cm.

Mortality was observed in the 9.6, 18 and 20 mg/L (10, 100 and 180 mg/L nominal) test chambers (see Table 51 below). Fish exposed to 9.6, 18, 16, 9.4, 18 and 20 mg/L of SAN 619F showed loss of equilibrium, dark discoloration, quiescence and tended to remain at the bottom of the test chamber. In addition, curved spine (cramps) was noted in the 16 mg/L test level (32 mg/L nominal). The at the lowest dose level (0.98 mg/L) the only affect noted was three fish on the bottom of the test chamber.

Table 51:   **Mortality in Rainbow Trout exposed to of SAN 619F over 96 hours (n = 10)**

Concentration [mg a.i./L]		24 hr	48 hr	72 hr	96 hr
Nominal	Measured	% Mortality	% Mortality	% Mortality	% Mortality
Water	-	0	0	0	0
DMF	-	0	0	0	0
1.0	0.98	0	0	0	0
10	9.6	0	0	0	10
18	18	0	0	0	0
32	16	0	0	0	0
56	9.4	0	0	0	0
100	18	0	0	0	10
180	20	0	60	90	100

Table 52:   **Acute toxicity of SAN 619F to Rainbow trout**

Endpoint	Endpoint value (mg/L)
Test object	Rainbow trout
24 hr LC$_{50}$*	> 20
48 hr LC$_{50}$*	>18

96 hr LC$_{50}$	19
Highest dose tested without significant effect (NOEC) 96 hrs	<0.98

* insufficient mortality for the statistical calculation of a reliable LC$_{50}$.

The 96 hour LC$_{50}$ was determined to be 19 mg/L (see Table 452 above). One hundred percent (100 %) mortality occurred at the highest dose level of 20 mg a.i./L (180 mg/L nominal) while only 10% died at the next highest dose level of 18 mg/L (100 mg/L nominal). The lowest dose level at which mortalities occurred was 9.6 mg/L (10 mg/L nominal). No mortalities or sub-lethal effects were observed at the lowest dose level of 0.98 mg/L, therefore the no observed effect concentration (NOEC) was determined to be <0.98 mg/L and the lowest observed effect concentration (LOEC) was 9.4 mg/L (56 mg/L nominal).

Acute Toxicity of SAN 619 F Technical to Bluegill Sunfish (Lepomis macrochirus); Bowman J.H. January 1988(b):

The acute toxicity of SAN619F technical to *Lepomis macrochirus* was investigated by Bowman J.H. in accordance with the *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988.*

In this static fish bioassay, bluegill sunfish (*Lepomis macrochirus*), 10 fish per test concentration, were exposed for 96 hours to nominal concentrations of 10, 18, 32, 56, and 100 mg SAN619F/L. Dimethylformamide (DMF) was used as the solvent (vehicle) to prepare the solutions (0.1 ml/L max.). An appropriate solvent control and dilution water control were run in parallel with the test concentrations. After 24 hours of testing an additional lower level (5.6 mg/L) was added along with another solvent control, in order to find a no-effect level. The stability of the test solutions was determined at 0 and 96 hours by gas chromatography. Temperature, pH and dissolved oxygen were also recorded at 0 hrs, 48 hrs and 96 hrs. At the initiation of the study, 10 fish were added to each test chamber by random assignment within 30 minutes after addition of test material. The average test chamber loading biomass was 0.21 g/L. Fish were observed for mortality and sub-lethal effects after 24, 48, 72, and 96 hours exposure and dead fish were removed and any observations recorded. The fish were not fed for the duration of the study and the test vessels were not aerated.

The mean measured test concentrations were 86, 92, 100, 75, 29 and 17% of nominal concentrations (4.8, 9.2, 18, 24, 16 and 17 mg/L of SAN 619F, respectively), therefore reported effects were based on mean measured concentrations. The pH values of the test waters ranged from 7.0 to 7.6. The dissolved oxygen concentrations ranged from 5.9 to 9.2 mg/L during the test, representing 70 and 108% saturation at 22 and 21°C respectively, which was considered adequate for testing, thus the chambers were not aerated. A precipitate was also observed during the study in the top four concentration levels (18, 32, 56 and 100 mg/L nominal), which increased with concentration, indicating that the water solubility may have been exceeded. The total hardness of the dilution water was 40-48 mg/L CaCO$_3$ and a total alkalinity of 25-35 mg/L CaCO$_3$.

Complete mortality, or 100% mortality, was observed at 24 mg/L (32 mg/L nominal) (see Table 53 below). Fish exposed to concentrations of 18, 16 and 17 mg/L of SAN 619F (18, 56 and 100 mg/L nominal values, respectively) showed loss of equilibrium, dark discoloration, quiescence and tended to stay at the bottom of the test chamber. Furthermore, occasional surfacing of the test fish was noted at the 17 mg/L SAN 619F (100 mg/L nominal) test concentration. Dark discoloration was the only affect noted at 9.2 mg/L SAN 619F (10 mg/L nominal) concentration mark. No abnormal effects were noted at the lowest dose level of 4.8 mg/L SAN 619F (5.6 mg/L nominal).

Table 53:   Mortality in Bluegill sunfish exposed to of SAN 619F over 96 hours (n = 10)

Concentration [mg a.i./L]		24 hr	48 hr	72 hr	96 hr
Nominal	Measured	% Mortality	% Mortality	% Mortality	% Mortality
Water	-	0	0	-	0
DMF 1	-	0	0	-	0
DMF 2	-	0	0	-	0
5.6	4.8	0	0	-	0
10	9.2	0	0	-	0
18	18	0	0	-	0
32	24	100	100	-	100
56	16	0	0	-	0
100	17	0	0	-	0

Table 54:   Acute toxicity of SAN 619F to Bluegill sunfish

Endpoint	Endpoint value (mg/L)
Test object	Bluegill sunfish
24 hr LC$_{50}$	21
48 hr LC$_{50}$	21
96 hr LC$_{50}$	21
LC$_{100}$	24
LC$_{0}$	18
Highest dose tested without significant effect (NOEC) 96 hrs	4.8

The 96 hour LC$_{50}$ of cyproconazole in the Bluegill sunfish was determined to be 21 mg/L (see Table 54 above). One hundred percent (100 %) mortality occurred at the highest measured dose level of 24 mg/L SAN 619F (32 mg/L nominal). No deaths occurred at any of the other dose levels, though various sub-lethal effects were noted at dose levels of 9.2 mg a.i./L (10 mg/L nominal) and above. No mortalities or sub-lethal effects were observed at the lowest measured dose concentration of 4.8 mg/L (5.6 mg/L nominal), therefore the NOEC was considered to be 4.8 mg/L and the LOEC was 9.2 mg/L.

SAN 619 F - Fish toxicity in the carp; Hamburger F. and Klotzsche C. July 1985:

The acute toxicity of SAN619F technical to *Cyprinus carpi* was investigated in accordance with the *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988* and the *OECD Guideline for the testing of chemicals: Fish, Acute Toxicity Test (OECD 203, 1992).*

In this static fish bioassay, carp (*Cyprinus carpio*), 10 fish per test concentration, were exposed for 96 h to nominal concentrations of 10, 12.5, 16, 20, 25, and 32 ppm SAN619F/L. Dimethylsulfoxide (DMSO) was used as the solvent (vehicle) to prepare the solutions (0.5 ml/L). An additional tank

containing 1 ppm SAN 619 F, without fish was run in parallel with the test concentrations, as well as an appropriate solvent control. The calcium content of the dilution water was 38.1 mg/L, the magnesium content was 9.4 mg/L. The pH ranged from 8.0 – 8.4. The stability of the test solutions was determined during the study but no report or analytical details were provided. Temperature, pH, dissolved oxygen were recorded after 2, 48 and 96 hrs. No details about the acclimatisation period were provided but fish were fasted for 48 hours prior to exposure. The average test chamber loading biomass was 0.83g/L. The behaviour of the fish was observed several times a day and mortality and sub-lethal effects were recorded after 24, 48, 72, and 96 hours exposure.

The mean measured concentrations were 90.8 to 98.4% of nominal value; therefore the observed effects were based on nominal concentrations. The pH values of the test waters ranged from 8.0 to 8.4, temperature ranged from 21.2 – 22.5 and the dissolved oxygen concentrations ranged from 8.05 to 8.4 mg/L during the test.

At 24hrs, all fish treated with 32 ppm had died (see Table 55 below) and 90% and 10% of the fish exposed to 25 and 20ppm, respectively, were dead. By 48 hours, one fish (10%) in each of the lower concentrations (10, 12.5 and 16 ppm) had also died. By 96 hours mortality at 20ppm increased to 20%. Signs of intoxication (including cramps, side position, supine position, dark colored, nystagmus, dorsal fin slack and weakness), were observed at all dose levels, the frequency increasing with concentration.

Table 55: **Mortality and sub-lethal effects in carp exposed to SAN 619F over 96 hours**

Nominal (ppm)	24 hr % Mortality	48 hr % Mortality	72 hr % Mortality	96 hr % Mortality	Observations
DMSO	0	0	0	0	NAD
1	n/a	n/a	n/a	n/a	n/a
10	0	10	10	10	7/9a; 1/9b; 9/9d; 4/9f; 10/10g
12.5	0	10	10	10	8/10a; 6/10d; 6/9f; 10/10g
16	0	10	10	10	10/10a; 2/9b; 9/9d; 8/10f; 10/10g
20	10	10	10	20	9/9a; 3/10b; 10/10d; 9/9f; 10/10g
25	90	90	90	90	9/10a; 8/10b; 10/10d; 1/1e; 5/10f; 10/10g
32	100	100	100	100	10/10a; 7/10b; 10/10d,6/10f; 10/10g

Observation key: No. of affected/no. in tank alive; **NAD** = no abnormalities detected; **n/a** = results not provided   ; **a** = cramps; **b** =side position; **c** = supine position; **d** = dark coloured; **e** = nystagmus; **f** = dorsal fin slack ; **g** =weak

Table 56: **Acute toxicity of SAN 619F to *Cyprinus carpi***

Endpoint	Endpoint value (ppm)
Test object	Carp
24 hr LC$_{50}$*	22.5
48 hr LC$_{50}$*	19.1
72 hr LC$_{50}$*	19.1

96 hr LC$_{50}$*	18.9
LC$_{100}$	29.1
LC$_0$	14.8**
96-h NOEC	< 9.6

* calculated based on mean measured values

** according to OECD 203 : 10% mortality allowed in the control group

Complete or 100 % mortality occurred at the highest measured dose level of 29.1 ppm SAN 619F (=32 ppm nominal) and at least 10% of fish died at all lower doses, including the lowest dose level of 9.6 ppm (10 ppm nominal). Based on mean measured concentrations, statistical analysis of data by binomial method gave a 96-h LC$_{50}$ of 18.9 ppm (see Table 56 above) for cyproconazole, in carp. From the same data, a re-calculation by Probit analysis (Litchfield-Wilcoxon) gave a LC$_{50}$ of 20 mg/L (95% Confidence Intervals: 17 – 23 ppm). The NOEC was considered to be <9.6 ppm as the LOEC was 9.6 ppm SAN 619F, the lowest dose tested.

Cyproconazole: a 96-hour flow-through acute toxicity test with the Sheepshead minnow (Cyprinodon variegatus); Drottar K.R. et al. September 1993:

The acute toxicity of cyproconazole technical to *Cyprinodon variegatus* was investigated by Drottar et al. in accordance with the *US-EPA Pesticide Assessment Guidelines, FIFRA Subdivision E § 72-1, Hazard Evaluation: Wildlife and Aquatic Organisms, October 1982. / ASTM standard E729-88, 1988.*

Sheepshead minnow (*Cyprinodon variegatus*), 10 fish per test concentration, were exposed for 96 hours under flow-through conditions to nominal concentrations of 6.5, 11, 18, 30, and 50 mg SAN619F/L. [^{14}C]-radiolabelled SAN 619F was added at a nominal radioactive concentration of 20 dpm/ml to allow for radiochemical determination of cyproconazole equivalent concentrations. Prior to exposure fish were acclimatized to laboratory conditions (temperature 20.1 – 24.9^{o}C) for 14 days with an acceptable level of mortality (0%). Dimethylformamid (DMF) (0.4 ml/L) was used as the solvent (vehicle) to prepare the solutions. The dilution water used throughout was the same filtered natural seawater (diluted with well water to a salinity of 20 ^{o}/$_o$; pH 7.7 – 7.9) as that used to maintain the fish stocks. Two additional groups were included as seawater and solvent controls. Two replicate test chambers were maintained for each treatment and control group, with 10 fish in each test chamber. All appropriate conditions (temperature, salinity, pH and dissolved oxygen) were measured continuously throughout the test period All test fish were acclimatised to the test conditions for 75 hours prior to test commencement and were not fed for the duration of the acclimatisation or study period.

Fish were observed for mortality and sub-lethal effects after 5.5, 24, 48, 72, and 96 hours exposure with any dead fish removed. The lethal concentrations (LC$_{50}$) were calculated by the moving average method (24 hrs) or by the binomial method.

1 below. Mean measured concentrations were 103, 95, 94, 87 and 68% of nominal concentrations (6.7, 10.4, 17, 26 and 34.1 mg/L of SAN 619F, respectively), therefore effects and LC$_{50}$ estimations were based on mean measured concentrations. The pH values of the test waters ranged from 7.9 to 8.4, temperature ranged from 21.8 to 22.1 and the dissolved oxygen concentrations remained above 60% saturation. Precipitate was observed in the mixing chambers and increased from low to high concentrations. This precipitate was the most probable explanation for the decrease in percent of nominal with increasing concentration.

At the 24hour-exposure mark, 14/20 fish at 26 mg/L and 19/20 fish at 34.1 mg/L test substance had died (see Table 57 below). All 20 fish at 26 mg/L had died by 72hrs, while all of the fish at the top dose level (34.1 mg/L) were dead by 48 hours. No mortalities occurred at any of the lower dose levels, though signs of intoxication (including lethargy and loss of equilibrium, discoloration, erratic swimming and moribund fish) were observed at 17 mg/L at various stages, from 24 hours onwards. Fish in the 6.7 and 10.4 mg/L treatment groups appeared normal throughout the test with no mortalities or overt signs of toxicity.

Table 57: Mortality and sub-lethal effects of SAN 619F on Sheepshead minnow recorded over 96 hours (n = 10 in all cases).

Concentration (mg/L)			5.5 hr		24 hr		48 hr		72 hr		96 hr	
Nominal	Actual	Replicate	% mortality	Effects	% mortality	Effects	% mortality	Effects	% mortality	Effects	% mortality	Effects
Water	-	A	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
		B	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
DMF	-	A	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
		B	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
6.5	6.7	A	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
		B	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
11	10.4	A	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
		B	0	10NAD	0	10NAD	0	10NAD	0	10NAD	0	10NAD
18	17	A	0	10NAD	0	2NAD; 5C; 3C,N;	0	1D,N; 4N; 5C	0	8NAD; 2E	0	5NAD; 2M; 3N
		B	0	NAD	0	6NAD; 4C	0	8NAD; 1D; 1N	0	9NAD; 1C	0	4NAD; 3M; 3N
30	26	A	0	8C; 1C, N; 1F, N	60	4C,N	90	1N,C	100	N/A	100	N/A
		B	0	5NAD; 4C; 1C,N	80	2C,N	100	N/A	100	N/A	100	N/A
50	34.1	A	0	7C, 2C,N; 1F,N	100	N/A	100	N/A	100	N/A	100	N/A
		B	0	2NAD; 5C; 3C,N	90	1C,N	100	N/A	100	N/A	100	N/A

Observation key : NAD = no abnormalities detected; N/A = not applicable; A = surfacing; C = lethargy; D = discoloration; E = erratic swimming; F = floating; M = moribund; N = loss of equilibrium

Table 58:   **Acute toxicity of SAN 619F to Sheepshead minnow**

Endpoint	Endpoint value (mg/L) (incl. confidence intervals)
Test object	Sheepshead minnow
24 hr LC_{50}*	24 (22-27)
48 hr LC_{50}**	22 (17-26)
72 hr LC_{50}**	21 (17-26)
96 hr LC_{50}**	21 (17-26)
LC_{100}	26
LC_0	17
96-h NOEC	10.4

*estimated via probit analyses

** estimated via binomial method

The 96 hour LC_{50} was determined as 21 mg cyproconazole/L (see Table 58 above). The lowest concentration that resulted in 100 % mortality during the study period was 26 mg/L. No mortalities or sub-lethal effects were observed at concentrations of 10.4 mg/L, therefore the NOEC and LOEC were determined as 10.4 mg/L and 17 mg/L respectively.

5.4.1.2 Long-term toxicity to fish

SAN 619 F - 21-day rainbow trout toxicity study under flow-through exposure conditions; Jenkins C.A. July 1989:

The chronic toxicity of SAN619F (cyproconazole) to rainbow-trout over a 21-day exposure period was investigated in accordance with the *OECD Guideline for the testing of chemicals: Fish prolonged toxicity test: 14-day study (OECD 204; 1984).*

Rainbow trout (*Oncorhynchus mykiss*), approximately 4 months old at study initiation with a mean body weight 1.34 g and average fork length of 5.2 cm, were exposed for 21 days under flow-through conditions to nominal concentrations of 0.75, 1.5, 3, 6, 12, and 24 mg SAN 619F (10 fish per dose level).

Test solutions were delivered to the test vessels by single axis siphon-dosers at a rate of 9.4 volume replacements every 24 hours. Stability of the test solutions was determined in duplicate on days 0, 7, 13 (12 mg/L), 14, 19 (24 mg/L) and 21 using GC. All test fish were held and observed for 14 days prior to testing with an acceptable level of mortality (< 1.1%). Each day of the test, fish were given an amount of proprietary trout pellets, equivalent to 2% of the total wet weight of the fish in each vessel. Mortality and behaviour of the fish were recorded within 20 minutes of their addition to the test vessels and daily thereafter (except for Day 6). At the end of the test, surviving fish were sacrificed and their individual wet weights and fork lengths were determined. The lethal concentrations (LC_{50}) were calculated at seven-day intervals using appropriate statistical methods (moving average).

Chemical analysis indicated that mean measured concentrations of SAN 619F for the 21-day study period were between 83 and 88% of nominal concentrations therefore effects were based on mean measured concentrations. A cream deposit was visible on the walls of the dosing apparatus at all exposure levels, although it was less evident at lower levels. Mortalities were observed at all concentrations except the lowest (0.645 mg/L), occurring within 1 day at the highest dose level (see Table 59 below).

Effects on behaviour: At measured concentrations of 1.25 mg/L and above the fish exhibited a loss of coordination typified by erratic swimming whilst orientated nose downwards. In the 5.04, 10.10 and 21.03 mg/L treatment groups, this loss of coordination became progressively more severe with time, rendering the fish completely immobile at the bottom of the test vessels. On day 10, dosing of test material at 21.03 mg/L failed for a short period but is not considered to have affected the validity of the test. The two fish remaining at this dose level had regained mobility and were swimming spirally in the dilution medium; approximately 23 hours after the re-establishment of dosing, the fish were once again immobile. Other effects observed throughout the test include darkened pigmentation, aggressive behavior towards other fish (active pursuit) and reduced activity with fish being subdued, resting on the bases of the vessels; these effects were not sustained throughout the test period and were not displayed by all of the fish. These symptoms were observed at all test concentrations except 0.65 mg/L.

Effects on fish growth (weight, length): The majority of the fish exposed to cyproconazole at 0.65 and 1.25 mg/L fed actively throughout the testing period. In the 2.50 and 5.04 mg/L treatment groups, feed consumption was reduced during the first 12 days of the study, thereafter some of the fish actively consumed the food. At 10.10 mg/L, the response of the fish was subdued and uneaten food was present on the base of the test vessel. At 21.03 mg/L the fish appeared to ignore the food provided for the duration of the test. On day 16 of the test the fish at 10.10 and 21.03 mg/L exhibited abdominal retraction; it is considered that starvation may have been a contributory factor to the death of fish at these higher levels. In the 10.10 mg/L treatment group, no effect on length was noticed while a bodyweight loss, due to starvation, was observed compared to controls (-14.3%). No effect on bodyweight and growth of surviving fish was reported up to 5.04 mg/L ($NOEC_{growth}$).

Table 59: Daily mortality of Rainbow trout following treatment with SAN 619F

Days	Control	Measured Concentration mg a.s./L					
		0.65	1.25	2.5	5.04	10.10	21.03
1	0	0	0	0	0	0	40
2	0	0	0	10	0	0	80
3 & 4	0	0	10	20	0	10	80
5	0	0	20	20	10	10	80
6	0	0	30	20	10	10	80
7	0	0	30	20	30	10	80
8	0	0	30	20	40	20	80
9	0	0	30	30	60	30	80
10	0	0	40	30	60	30	80
11	0	0	40	30	70	30	80
12	0	0	50	30	70	30	80
13 & 14	0	0	50	30	70	30	80
15	0	0	50	30	70	40	90
16	0	0	50	30	70	40	90

17 & 18	10	0	50	30	70	50	90
19	10	0	50	30	70	50	100
20	10	0	60	30	70	50	100
21	10	0	70	30	70	50	100

The 7, 14, and 21-day LC_{50} values, based on measured concentrations, are presented below (Table 60 below).

Table 60: Chronic toxicity of SAN 619F to rainbow trout

Endpoint	Endpoint value (mg/L) based on mean measured concentrations
7-day LC_{50} (and 95% confidence interval)	15.55 (12.1 – 21.5)
14-day LC_{50} (and 95% confidence interval)	4.56 (2.84 – 8.46)
21-day LC_{50} (and 95% confidence interval)	3.20 (2.19 – 4.55)
LC_{100}	21.03
LC_0	0.65
21-day NOEC	0.65
21-day NOEC (growth)	5.04

The highest nominal concentration at which no mortality occurred and the lowest at which there was 100% mortality were 0.65 and 21.03 mg SAN 619F, respectively. Based on mean measured concentrations, the 21-day LC_{50} for SAN 619F in rainbow trout was determined to be 3.20 mg/L. The lowest concentration at which slight clinical effects were observed was 1.25 mg/L, hence the NOEC was considered to be 0.65 mg SAN 619F/L.

Cyproconazole (SAN 619): an early life-stage toxicity test with the Rainbow trout (Oncorhynchus mykiss), Drottar, K.R. and Swigert, J.P. December 1993(a):

The ability of SAN619F (cyproconazole) to induce early life-stage toxicity in rainbow-trout was investigated in accordance with the *U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-4(a) (1982), ASTM Standard E 1241-88 (1988), and U.S. EPA "Standard Evaluation Procedure, Fish Early Life-Stage Test" (1986).*

Rainbow trout embryos were exposed to five nominal test concentrations of 0.15, 0.30, 0.60, 1.2 and 2.4 mg cyproconazole/L under flow-through conditions for a total of 89 days (27-day hatching period and 62-day post-hatch period). An appropriate solvent control (Dimethylformamide (DMF)) and dilution water control were run in parallel with the test concentrations. Four replicate test chambers were maintained in each treatment and control group with each test chamber containing two incubation cups. Each cup contained 15 embryo's resulting in a total of 30 embryos per replicate and 120 embryos per dose group. The test was initiated within four hours of fertilisation, with the impartial distribution of newly fertilised eggs to the incubation cups. Fifteen embryos were held in each of four extra incubation cups in dilution water and were sacrificed on Day 11 to evaluate fertilisation success. After ≥95% of the controls had reached the swim up stage, the number of larvae in each replicate was reduced to 15 to prevent overcrowding.

Survival and growth were monitored over the total period of 89 days. The criteria for effects included hatching success of embryos, time to hatch, time to swim up of the larvae, post hatch growth (length, weight) and survival of juveniles. Post hatch percent survival was calculated for two intervals, prior to (days 0 – 18 post hatch) and after thinning (days 18 – 62 post hatch). Post hatch growth was measured on day 32 post hatch and at the end of the test.

Analytical results:

Chemical analysis indicated that the mean measured concentrations of SAN 619 F for the study period represented 106, 97, 97, 92 and 96 % of the nominal concentrations of 0.15, 0.3, 0.6, 1.2 and 2.4, respectively). The mean measured concentrations were used to express the LOEC, NOEC and MATC, when possible. ^{14}C-cyproconazole accounted for approximately 98% of the radioactivity in the test solutions. During the study the water temperature was maintained at 11.8-12.9^{o}C, the dissolved oxygen ranged from 7.2 to 9.9 mg/L and pH from 8.1-8.4. The total hardness of the dilution water ranged from 136-152 mg/L $CaCO_3$, total alkalinity ranged from 182-194 mg/L $CaCO_3$ and conductivity ranged from 280 to 320 µmhos/cm.

General observations:

During the test, it was noticed that some fish in the treatment groups had developed an extended lower jaw. Fish in the negative control, solvent control, and 0.16 mg/L treatment groups did not show this deformation. One of 60 surviving fish in the 0.29 mg/L treatment group showed this malformation, whereas all remaining fish in the 0.58 (58/58) and 1.1 (26/26) mg/L treatment groups showed the malformation to some extent. Due to the concentration-response relationship observed, this malformation was considered to be treatment-related.

Percent fertilisation:

Mean percent fertilization (egg viability) was 93.3%.

Hatching success and time to hatch:

The rainbow trout embryos began hatching on Day 24 and continued until Day 27. Hatching success in all of the treatment groups was compared to the solvent control (51%), as it was statistically significantly lower than the hatching success in the water control group (67%). Hatching success in the treatment groups ranged from 59 – 68% (see Table 57 below) and therefore was not considered to have been affected by exposure to cyproconazole.

Time to swim up:

The rainbow trout began swimming up at 12 days post hatch (Day 39). By Day 17 post hatch (day 44), all of the control organisms and all of the fish at the 0.16 and 0.29 mg/L test concentrations had completed the swim up stage of development, whereas only 14%, 1% and 0% of the surviving fish had completed swim up at 0.58, 1.1 and 2.3 mg/L, respectively. Tabulated swim up data was only provided up until 17days post hatch (day 44), but according to the summary provided in the report, by Day 24 post hatch (day 51) only 56% of the larvae in the1.1 mg/L group had attained swim up and larvae in the 2.3 mg group never attained swim up (no data on what happened in the 0.58 mg/L group after day 17 post hatch). Therefore time to swim up was considered to be treatment related as it was increased in the 1.1 and 2.3 mg/L treatment groups.

Larvae and fry survival:

Survival in all of the treatment groups was compared to survival in the solvent control as it was slightly lower (95% before and after thinning) compared to survival in the water control group

(99% before thinning, 100% after thinning) (see Table 61 below). Survival in the 0.16, 0.29, 0.58, 1.1 and 2.4 mg cyproconazole/L treatment groups averaged 97, 98, 95, 98, and 67% respectively, before thinning (days 0 – 18 post hatch), and averaged 100, 100, 97, 45 and 0% respectively, after thinning (days 18 – 62 post hatch). Statistical analyses indicated that the reduction in survival was treatment related in the 2.3 mg/L treatment group before thinning and in the two highest exposure levels (1.1 and 2.3 mg/L) after thinning (days 18 – 62 post hatch).

Growth:

Effects on growth in the treatment groups were compared to the pooled controls in this case as there was no statistical difference in growth measurements between the water and solvent controls. When measured at 32 days post hatch (*i.e.* on day 59 of the study), length was decreased in the 0.58 and 1.1 mg/L treatment groups (see Table 61 below). When measured at test termination, length, dry weight and wet weight in all cyproconazole treatment groups were statistically reduced. The decreased growth appeared to be directly related to the treatment concentration.

Table 61:   Toxicity of SAN 619F to the early life stage of the Rainbow trout

Parameter measured	Water control	Solvent control	Concentration (mg/L)				
			0.16	0.29	0.58	1.1	2.3
Hatching Success (%)	67	51	63	63	68	59	63
Survival days 0 – 18 post hatch (%)	99	95	97	98	95	98	67
Survival days 18 – 62 post hatch (%)	100	95	100	100	97	45	0
Mean length (mm), day 32 post hatch	31.6	31.0	31.1	30.2	29.6*	26.2*	22.1*
Mean length (mm), day 62 post hatch	48.0	48.0	44.6*	44.6*	44.3*	40.1*	-
Wet weight (mg), day 62 post hatch	1116	1128	873*	829*	791*	571*	-
Dry weight (mg), day 62 post hatch	215	234	161*	153*	147*	101*	-

*   statistically significant (P ≤ 0.05) compared to controls.

A summary of the relevant NOEC's for all of the significant effects of SAN 619F on the early life stage of rainbow trout is provided in Table 62, below. Growth was the most sensitive biological parameter.

Table 62:   List of relevant endpoints for all of the significant effects of SAN 619F on the early life stage of Rainbow trout.

Endpoint	Endpoint value (mg SAN 619F/L) based on mean measured concentrations
NOEC for time to swim up	0.58
LOEC for time to swim up	1.1
NOEC for larvae and fry survival	0.58
LOEC for larvae and fry survival	1.1
NOEC for growth	< 0.16
LOEC for growth	0.16
NOEC for clinical developmental effects	0.16

LOEC for clinical developmental effects	0.29

Exposure of rainbow trout to SAN 619F to doses up to 2.3 mg/l during its early life stage had a significant effects on various biological parameters, namely the time it took to attain swim up, larvae and fry survival and growth. Early exposure to SAN 619F also caused deformities (extended lower jaw) at quite low dose levels (0.29 mg/L and above). The most sensitive biological parameter was growth, with fish length, wet weight and dry weight all being statistically reduced at study termination in all treatment groups, relative to controls. Therefore the worst-case NOEC and LOEC for rainbow trout exposed to SAN619F (cyproconazole) in their early life stage were considered to be 0.16 mg/l and < 0.16 mg/l respectively.

CYPROCONAZOLE (SAN 619): Early Life-Stage Toxicity to RainbowTrout (Oncorhynchus mykiss); Wheeler, J.R. (2006)

The objective of this study was to determine the No-Observed-Effect Concentration (NOEC) of cyproconazole to Rainbow Trout (*Oncorhynchus mykiss*) embryos and larvae under flow-through conditions following OECD Guideline 210 and the *US-EPA Ecological Effects Test Guidelines OPPTS Draft Guideline 850.1400 Fish Early-Life Stage Toxicity Test.*

Rainbow trout (*Oncorhynchus mykiss*) embryos (less than 24 hrs old) were exposed to six nominal test concentrations of 9, 19, 38, 75, 150 and 300 µg cyproconazole/L, and a dilution water control (and a fertilisation control) under flow-through conditions. At the start of the test 30 eggs were randomly allocated to egg cups and one egg cup suspended in each of three replicate test vessels at each test and control treatment (including fertilisation control). Therefore, 90 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath. Eggs and fry were exposed to mean measured concentrations of 5.59, 18.8, 39.1, 72.2, 160 and 305 µg/L, dilution water and fertilisation control.

Observations for time to hatch, hatching success, larval mortality, deformed larvae and other symptoms of toxicity were made daily during the pre and post-hatch phases, as appropriate. At the end of the test, lengths and wet weights of the surviving fry were measured. No Observed Effect Concentrations (NOECs) were estimated from the data obtained.

The egg viability of the fertilisation control was 88% (determined after 14 days). Survival at hatching in the three dilution water control replicates ranged from 83-93% and from 70-93% in the cyproconazole treatments. There was no statistically significant (two-tailed Fisher's exact test) difference between dilution water control and any cyproconazole treatment for survival at hatching.

Larval survival in the three dilution water control replicates ranged from 96-100% and from 81-100% in the cyproconazole treatments. There was no statistically significant (two-tailed Fisher's exact test) difference between dilution water control and any cyproconazole treatment for survival between hatching and test termination Hatching started synchronously in all replicates of the dilution water control and cyproconazole treatments on day 34 ("Hatching day") and was complete by Day 35. Swim-up began on Day 47 and was complete by Day 56 in all treatments.

Table 63: **Effects of Cyproconazole (SAN 619) technical on the survival and growth of early life stage of Rainbow trout (*Oncorhynchus mykiss*).**

Mean measured concentration (µg ai/L)	Quantal responses			Non quantal responses	
	Mean hatching	Mean survival at hatch (%)	Mean survival (%) day	Mean length (mm)	Mean wet weight

	success (%)	day 0-34	34-93		(mg)
Control	84	86	97	49	985
5.59	76	76	91	48	960
18.8	86	86	95	49	964
39.1	88	88	91	48	922
72.2	81	81	95	50	1015
160	73	72	96	49	963
305	85	85	95	48	831

There were no statistically significant effects of cyproconazole on hatching success, survival or larval growth. Therefore, the overall study NOEC is 305 µg cyproconazole/L based on mean measured concentrations.

CYPROCONAZOLE – Fish Full Life-Cycle Test with Fathead Minnow (Pimephales promelas); Carfarella, M.A. (2009):

The chronic toxicity of cyproconazole technical on the life-cycle of the fathead minnow *Pimephales promelas* was investigated in accordance with the *US-EPA, Fish Life Cycle Toxicity Tests OPPTS 850.1500 Draft Guideline. The test protocol was adapted to allow the assessment of additional endpoints (gonadal histopathology and blood plasma vitellogenin levels).*

Approximately 1700 embryos (equally divided into eight embryo incubation cups (two cups in each of four replicate aquaria) per exposure level and the control) were continuously exposed to five concentrations of cyproconazole and a dilution water control for a complete life-cycle (total 263 days), including exposure of progeny (F_1) until approximately 90 days post-hatch. All exposure levels were maintained in quadruplicate. The nominal exposure concentrations of cyproconazole selected for the study were 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.i./L. Samples of the exposure solutions were analysed for cyproconazole weekly throughout the exposure period.

The biological endpoints evaluated were first generation (F_0) hatching success, survival, growth (total length and wet weight) and reproduction (eggs/female, spawns/female, eggs/spawn), histological sex ratio as well as plasma vitellogenin concentration (VTG) and gonad histopathology; second generation (F_1) hatching success, survival, growth, histological sex ratio, plasma vitellogenin concentration (VTG) and gonad histopathology.

Analytical results:

The analytical results demonstrate that measured concentrations at each exposure level were generally consistent over the 263-day exposure and the desired 50% dilution series was maintained throughout the study. Based on these analyses, the mean measured exposure concentrations of cyproconazole were 0.060, 0.14, 0.25, 0.51 and 0.95 mg a.i./L, ranging from 95 to 110% of the nominal concentrations.

During the study the water temperature was maintained between 24.0 ^{o}C and 26 °C, pH ranged from 6.6 to 7.8, alkalinity ranged from between 18 to 26 m/L while conductivity was between 180-270 µmhos/cm.

Reproduction:

Table 64: **The effects of Cyproconazole on the Reproductive Performance in the F_0 generation of Fathead minnows**

Mean Measured Concentration (mg a.i./L)	Reproductive performance		
	Number Eggs/ Spawn (SD)	Number Spawns/ Female (SD)	Number Eggs/ Female (SD)
Control	144 (22)	20.7 (2.5)	2957 (315)
0.06	137 (13)	15.1 (1.8)	2072 (388)
0.14	160 (7)	23.2 (3.9)	3734 (729)
0.25	167 (23)	18.1 (3.1)	3082 (962)
0.51	166 (20)	18.4 (3.2)	3014 (327)
0.95	181 (20)	14.5 (0.68)*	2632 (350)

* Significantly reduced compared to the control, based on Williams' test.

Survival:

Table 65: **The effect of Cyproconazole on survival of the F_0 and F_1 generations of Fathead minnows (expressed as % hatching success, larval survival and lengths 30 and 60 days post hatch)**

Nominal Conc.(mg a.i./L)	F_0 (30 dph)			F_1 (30dph)			F_0 (60 dph)		F_1 (60dph)	
	Embryo Hatching Success (%)	Larval Surv. (%)	Mean Total Length (mm)	Embryo Hatching Success (%)	Larval Surv. (%)	Mean Total Length (mm)	Larval Surv. (%)	Mean Total Length (mm)	Larval Surv. (%)	Mean Total Length (mm)
Control	94 (5)	90 (6)	29.7 (2.55)	93 (10)	95 (6)	28.6 (3.55)	89 (5)	43.6 (4.25)	94 (9)	41.6 (5.52)
0.06	90 (7)	92 (4)	29.4 (2.66)	95 (6)	91 (9)	28.5 (2.77)	92 (4)	43.7 (4.61)	87 (9)	44.0 (4.84)
0.14	85 (9)	97 (5)	30.3 (2.88)	93 (10)	98 (4)	28.9 (2.00)	96 (4)	43.6 (3.82)	98 (4)	42.5 (2.81)
0.25	90 (6)	93 (2)	30.1 (2.93)	98 (4)	94 (12)	28.4 (2.75)	93 (1)	43.8 (4.15)	93 (15)	41.6 (3.11)
0.51	86 (6)	97 (2)	30.1 (3.06)	94 (6)	95 (4)	29.1 (3.01)	94 (3)	44.0 (3.81)	94 (4)	43.0 (4.16)
0.95	90 (6)	97 (3)	29.5 (2.96)	95 (4)	88 (8)	27.9 (2.20)	96 (4)	43.8 (3.81)	88 (8)	42.8 (2.92)

Dph = days post hatch;

Table 66: **The effect of Cyproconazole on survival of the F_0 and F_1 generations of Fathead minnows (expressed as % survival, mean total length and mean wet weight 90 days post hatch)**

Mean Measured Concentration (mg a.i./L)	Survival (%)		Mean Total Length (mm) F_0		Mean Wet Weight (g) F_0		Mean Total Length (mm) F_1		Mean Wet Weight (g) F_1	
	F_0	F_1	Male	Female	Male	Female	Male	Female	Male	Female
Control	88	93	56.6	47.4	2.12	1.21	53.1	43.3	1.94	1.03
0.06	91	84	56.8	47.9	2.07	1.21	56.1	48.4	2.13	1.30
0.14	95	98	56.3	48.8	2.00	1.25	55.7	47.4	1.96	1.22
0.25	92	93	55.0	48.9	1.88	1.28	55.1	46.9	1.95	1.18
0.51	94	92	57.8	49.7	2.06	1.28	56.3	48.8	2.02	1.27
0.95	95	88	57.4	49.6	1.88	1.29	57.8	48.8	2.09	1.31

Table 67: **The effect of Cyproconazole on survival of the F_0 generations of Fathead minnows (expressed as % survival, mean total length and mean wet weight 213 and 263 days post hatch)**

Mean Measured Conc. (mg a.i./L)	Survival (%)	Mean Total Length (mm) F_0		Mean Wet Weight (g) F_0		Mean Total Length (mm) F_0		Mean Wet Weight (g) F_0	
		Male	Female	Male	Female	Male	Female	Male	Female
		Non-spawning F_0 213 days post-hatch				Spawning fish F_0 263 days post-hatch			
Control	98	68.0	53.0	3.93	1.72	65.7	53.8	3.91	1.63
0.06	96	68.0	54.2	4.01	1.84	69.5	54.4	4.39	1.82
0.14	98	69.7	54.2	4.13	1.81	68.1	55.1	4.07	1.78
0.25	96	70.3	55.4	4.18	1.97	67.4	55.6	3.62	1.78
0.51	95	70.5	57.0	4.19	2.01	68.2	56.5	3.91	1.78
0.95	99	72.2	58.7	4.21	2.21	68.9	58.2	4.19	1.96

Biomarkers of endocrine effects:

This study incorporated biological measurements (vitellogenin) to facilitate the detection of endocrine disrupting substances. This endpoint has been proposed by the *OECD Endocrine Disruptors Testing and Assessment (EDTA) Task Force (2001)*.

Table 68:	Vitellogenin analysis – Male F_0	Vitellogenin analysis – Male F_1	Vitellogenin analysis – Female F_0	Vitellogenin analysis – Female F_1

The effect of Cyproconazole on Vitellogenin F_0 and F_1 generations of Fathead minnows 90 days post hatch Mean Measured Conc. (mg a.i./L)	Treatment Mean (ng/mL)	(%) Reduction	Treatment Mean (ng/mL)	(%) Reduction	Treatment Mean (ng/mL)	(%) Reduction	Treatment Mean (ng/mL)	(%) Reduction
Control	168	NA	529	NA	518974	NA	621750	NA
0.06	134	20.4	366	30.7	798504	-53.9	480152	22.8
0.14	105	37.8	266	49.8	596035	-14.9	81973	86.8
0.25	52	69.1	203	61.6	648347	-24.9	121992	80.4
0.51	114	32.3	446	15.6	284129	45.3	148509	76.1
0.95	106	37.1	35	93.5	121634	76.6	254270	59.1

A summary of the NOEC values for the effects observed for both the F_0 and F_1 generation areummarized in Table 69 below.

Table 69:   A summary of the relevant NOEC's for the effects of Cyproconazole technical on various stages of the life cycle of Fathead minnows

Endpoint	NOEC [mg ai/L]
F0 Generation	
F0 percent hatch	0.95
F0 time to hatch	0.95
F0 30-day survival	0.95
F0 30-day total length	0.95
F0 60-day survival	0.95
F0 60-day total length	0.95
F0 90-day survival	0.95
F0 90-day male length	0.95
F0 90-day male wet weight	0.95
F0 90-day female length	0.95
F0 90-day female wet weight	0.95
F0 90-day VTG concentration	0.95
F0 90-day histological sex ratio	0.95
F0 216-day survival	0.95

Endpoint	NOEC [mg ai/L]
F0 male length at maturation	0.95
F0 male weight at maturation	0.95
F0 female length at maturation	0.95
F0 female weight at maturation	0.95
F0 termination (Day 263)	
F0 male total length	0.95
F0 male wet weight	0.95
F0 female total length	0.95
F0 female wet weight	0.95
F0 eggs/female	0.95
F0 spawns/female	0.51
F0 eggs/spawn	0.95
F0 overall histological sex ratio	0.95
F1 Generation	
F1 percent hatch	0.95
F1 time to hatch	0.95
F1 30-day survival	0.95
F1 30-day total length	0.95
F1 60-day survival	0.95
F1 60-day total length	0.95
F1 90-day survival	0.95
F1 90-day male total length	0.95
F1 90-day male wet weight	0.95
F1 90-day female total length	0.95
F1 90-day female wet weight	0.95
F1 90-day VTG concentration	0.95
F1 histological sex ratio	0.95

Conclusion:

No internal deformities were observed at any of the treatment levels tested. The sex of fish was histologically confirmed for sex ratio determinations. No adverse histopathological findings were observed

Based on mean measured concentrations, the NOEC for cyproconazole was 0.51 mg a.i./L. However, as the effect on the number of spawns/female was marginal and not consistent with other population-relevant reproductive endpoints, the overall NOEAC is 0.95 mg a.i./L after 263 days.

CYPROCONAZOLE tech. (SAN 619): Determination of effects on the life cycle of the fathead minnow (Pimephales promelas), including measurements of vitellogenin and gonad histopathology; Caunter, J.E. and Williams, T.D. July 2001:

The effects of SAN619F (cyproconazole) on the life-cycle of the fathead minnow *Pimephales promelas* was investigated in accordance with the *US-EPA, Fish Life Cycle Toxicity Tests, EPA*

540/9-86-137, July 1986, incorporating biological endpoints proposed by the *OECD Endocrine Disrupters Testing and Assessment (EDTA) Task force.*

F_0 Fathead minnow (*Pimephales promelas*) embryos (less than 24 hrs old) were exposed to five nominal test concentrations of 0.125, 0.25, 0.50, 1.0 and 2.0 mg cyproconazole/L under flow-through conditions. An appropriate solvent control (DMF) and dilution water control were run in parallel with the test concentrations. Four replicate test chambers were maintained in each treatment and control group with each test chamber containing two incubation cups. Each cup contained 25 randomly selected and newly fertilized fish eggs resulting in a total of 50 eggs per replicate and 200 eggs per dose group (loading = 5.3 eggs per litre of test solution; nominal flow loading = 60 ml per egg per hour).

Egg mortality and hatching in each incubation cup was recorded daily. After hatching (on day 4) the fry were impartially reduced to 40 per replicate (i.e. 160 fry per concentration) and released into progeny tanks. Effects on survival, growth (weight, length), reproduction parameters, vitellogenin (VTG) and gonad histopathology were investigated. Any abnormal sub-lethal changes of eggs and fry were observed on 2 generations (F_0 and F_1). Daily observations were made of mortality, behaviour and appearance and any abnormalities recorded. On day 60 (56 days post hatch) all surviving fish were photographed for length. Surviving fish were transferred to each of the duplicate adult tanks, giving a maximum of 80 fish per tank. On days 92 and 93 post hatch 40 fish from each tank were sacrificed for VTG analysis and gonad histopathology (key biomarkers of reproductive effects in fish).

The remaining fish were photographed and impartially reduced to 25 per tank. On day 151 paired males and females were introduced into each of 4 breeding chambers per tank. Over days 152-172 any non-compatible breeding pairs were replaced from the retained fish. On day 173 remaining adults were sacrificed and weight, length, VTG and gonad histopathology. Spawning occurred from day 158 to 177. For each duplicate tank spawnings of at least 50 embryos from single females were used for hatchability trials and early life stage (ELS) tests. F_0 exposure was terminated on day 301 of the study (day 297 post hatch). Two early life stage toxicity tests were performed on the F_1 generation: F_{1E1} and F_{1E2}. The F_{1E2} test was terminated after 56 days post hatch and the F_{1E1} test 93 days post hatch to facilitate gonad histopathology.

Analytical results:

Mean measured values ranged from 97 – 112% of nominal concentrations in adult tanks and from 96 – 104% of nominal concentrations in the progeny tanks. Therefore, nominal concentrations were used in the reporting of results.

During the study the water temperature was maintained between 24.0 °C and 25.5°C, pH ranged from 7.02 to 7.97 and mean dissolved oxygen content between 4.0 and 8.8 mg/L (corresponding to 49 – 107% ASV).

Survival:

Statistical analysis of the F_0 and F_1 survivorship data is shown in Table 70 below. Compared to pooled controls, a significant reduction in survivorship was observed in the F_0 fish 92/93 days post hatching exposed to nominal concentrations of 2 mg/L. No adverse effects on survivorship were observed in the F_1 generation of fish.

Table 70: The effect of cyproconazole on survival of the F_0 and F_1 generations of Fathead minnows (expressed as % survival in pooled replicates on the days indicated)

Nominal Concentration	F_0		F_1	
(mg/L)	56 dph	92/93 dph	56 dph	92/93 dph
Water control	95	96	94	87
Solvent control	96	97	91	80
0.125	86**	96	96	95
0.25	98	98	90	96
0.5	97	96	91	87
1.0	99	99	88	98
2.0	90	90*	87	97

Dph = days post hatch;

* significant reduction (P = 0.05) in survivorship compared to pooled dilution water control and solvent control

** statistical result was not considered biologically relevant

Growth:

There was no significant effect on length or weight of F_0 or F_1 fish exposed to cyproconazole, compared to fish in the dilution water control, the solvent control or the pooled controls (see Table 71) below. Therefore the NOEC and LOEC for effects of cyproconazole on growth in fathead minnow were considered to be 2 mg/L and > 2 mg/L, respectively.

Table 71: The effect of cyproconazole on growth of the F_0 (92/93 dph) and F_1 generations (56 and 92/93 dph) of Fathead minnows (expressed as mean length and weight of pooled replicates). No differentiation was made between sex of fish

Nominal Concentration (mg/L)	F_0 generation			F_1 generation		
	n	Length (mm)	Weight (g)	n	Length (mm)	Weight (g)
Water control	80	38.1	1.10	79	40.9	1.43
Solvent control	80	38.8	1.17	71	42.5	1.54
Combined water & solvent control	160	38.5	1.13	150	41.7	1.48
0.125	76	38.8	1.16	80	43.1	1.60
0.25	80	40.0	1.26	96	43.0	1.61
0.5	80	38.6	1.14	85	43.7	1.62
1.0	80	40.4	1.28	81	44.1	1.59
2.0	80	39.4	1.22	66	44.0	1.78

Reproduction:

Egg production in fathead minnow was characterized by high individual variability between individuals within a treatment, as indicated by the magnitude of the standard deviations (see Table

72). As a consequence of this variability, the statistical procedures are less powerful for detecting significant differences between treatments compared to egg hatchability data, which was considered to be a more reliable endpoint than egg production.

Egg production:

Despite rigorous cleaning of exposure tanks, excessive microbial growth was observed in all exposure tanks receiving the DMF solvent carrier. Egg production in tanks receiving the DMF therefore may have been influenced by (a) bad water quality (due to excessive microbial growth), (b) the solvent itself directly, or (c) an interaction between the solvent, microbial growth and different concentrations of the test substance. Nonetheless, statistical comparisons between treatments and the solvent control data were considered to be the most relevant, as the solvent control and all exposure treatments had the same level of solvent. Compared to the solvent control there were no significant effects on egg production in the F0 generation at exposure concentrations up to and including the highest exposure level (NOEC and LOEC of 2 mg/L and > 2 mg/L, respectively), though only 2 of the 8 female fish at the highest dose level of 2 mg/L, produced eggs. Possible reasons why significant differences were not found may include the high within treatment variability in egg production and a requirement to use non-parametric (less powerful) statistical procedures to analyse the data.

Taking into account the poor reproductive performance in the solvent control, statistical comparisons were also made between treatments and the dilution water control. In this case (compared with the dilution water control), there was a significant reduction in egg production at concentrations of 0.25 mg/L and higher, (NOEC and LOEC of 0.125 mg/L and 0.25 mg/L), respectively as summarized in Table 60 below, though, as noted above, there was considerable intra-individual variability in egg production even within treatments, resulting in large standard deviations. It should be borne in mind also that egg production in the solvent control was also significantly lower than in the dilution water control.

In light of these conflicting statistical datasets, the egg production data in this study was compared with historical control data from other fish life cycle studies with fathead minnow (Table 73 below). Statistical analysis of this data indicated that production of eggs by the fish in the dilution water control tanks in this study, was not significantly different from two previous studies, but was significantly higher than in data from 4 previous studies, verifying that the egg production by the dilution water control batch in this study, was suitable.

By comparison, egg production by solvent control fish was poor but comparisons with historical control data indicate that the values recorded in this study were not significantly different from that recorded in 2 previous studies, but was significantly lower than that from a third study (Table 74 below). However, these comparisons should be treated with caution as they include a different choice of solvent and varying solvent levels (Table 73). Despite these differences, these datasets demonstrate the variable nature of egg production and how it can be potentially influenced by both solvent type and solvent concentration.

Egg hatchability:

Compared with pooled controls, there were no significant effects on F0 egg hatchability at any of the exposure levels and no effects on the F_1 egg hatchability up to and including 0.5 mg/L (Table 72 below). Within the F_1 generation, at nominal concentrations of 1.0 and 2.0 mg/L, only 4 females produced batches of eggs from which hatchability trials were conducted. Of the 4 hatchability trials carried out at these exposure levels, there were only three successful egg hatches, thus there was insufficient data to perform statistical analyses. Based on the available data, the NOEC and LOEC for hatchability within the F_1 generation, are considered to be 0.5 and > 0.5 mg/L, respectively,

though these values are more likely a reflection of the poor egg production at 1.0 and 2.0 mg/L rather than effects on hatchability per se.

Table 72: **The effect of cyproconazole on egg production (expressed as mean no. of eggs per individual fish for pooled replicates) and hatchability (expressed as mean % hatch of eggs in each treatment) of the F_0 and F_1 generations of fathead minnows**

Nominal Concentration (mg/L)	Egg production (F_0 generation)		Hatchability (F_0 and F_1 generation)			
	F_0 (no. eggs ± std. dev.)	n	F_0 generation (% hatch of eggs in each treatment)	n	F_1 generation (% hatch of eggs in each batch taken from individual fish)	n
Water control	2494 ± 1095	8	98	8	90	16
Solvent control	325* ± 444	8	98	8	93	6
0.125	1386 ± 1174	8	97	8	88	12
0.25	403* ± 230	8	94	8	92	10
0.5	808* ± 937	8	97	8	94	9
1.0	257* ± 374	8	96	8	-	3
2.0	79*± 180	8	97	8	-	1

* significant difference (P = 0.05) compared to the dilution water control

Table 73: **Historical control data for egg production in fathead minnows exposed to dilution water.**

Dilution water control data identified by study date	Study No.	Report No.	Mean total no. of eggs produced by individual female fish	Standard Deviation (±)
This study	AG0397/A	BL7106/B	2494	1095
3/88-1/89	Q/600/E	BL3476/B	1552	1105
6/89-6/90	S001/A	BL3737/B	352*	345
7/95-6/96	AA1099/B	BL5711/B	724*	756
10/95-9/96	AB0450/A	BL5728/B	1036*	591
11/98-02/00 (F0)	AF0567/A	BL6878/B	794*	812
11/98-02/00 (F1)	AF0567/A	BL6878/B	4103	2784

* significantly lower no. of eggs (P = 0.05, Steel's Many One-Rank Test) compared with data from this study

Table 74: **Historical control data for egg production in fathead minnows exposed to solvent control media, as indicated.**

Solvent control data identified by study date	Solvent type and Concentration (µL/L)	Study No.	Report No.	Mean total no. of eggs produced by individual female fish	Standard Deviation (±)
This study	DMF (100, days 0 – 210) 20, days 213 – end)	AG0397/A	BL7106/B	325	444
3/88-1/89	Trigol (12.5)	Q/600/E	BL3476/B	1407	1455
6/89-6/90	Trigol (1.357)	S001/A	BL3737/B	214	202
10/95-9/96	Trigol (71)	AB0450/A	BL5728/B	1284*	1023

* significantly higher no. of eggs (P = 0.05, Steel's Many One-Rank Test) compared with data from this study

<u>Biomarkers of endocrine effects:</u>

This study incorporated biological measurements (vitellogenin and gonad histopathology) to facilitate the detection of endocrine disrupting substances. These endpoints have been proposed by the *OECD Endocrine Disruptors Testing and Assessment (EDTA) Task Force (2001)*. Sex determination of these fish was established by gonad histopathology prior to measurement of VTG concentration, therefore, the data for male and female fish could be analysed separately.

In general, there were significant differences in VTG concentrations between fish from the dilution water controls and solvent controls. The reasons for these differences are unknown but may have been due to the solvent carrier, therefore, comparisons with the test substance treatments were made using the solvent control data only.

Vitellogenin concentration - males:

There were no significant increases in VTG concentration in male fish of the F_0 generation at any dose level, compared to solvent controls (Table 75 below). In the F_1 generation, there were one or two fish in each of the solvent control and the 0.25 and 0.5 mg/L concentrations, which produced unusually high VTG concentrations (more than 100 times higher than the LOD). However, apart from these few fish, VTG concentrations in male fish at all treatments were quite low. It was concluded therefore, that, relative to the solvent control, there were no significant increases in VTG concentrations in male fish of the F_1 generation when exposed to cyproconazole.

Vitellogenin concentration - females:

Compared to the solvent control, there was a significant decrease in VTG concentrations of 1.0 mg/L and above in female fish of the F_0 generation (Table 75 below). The reason for this decrease is unknown, though the study author suggested that it may have been caused by the DMF solvent increasing the metabolism or excretion of endogenous estradiol. However, similar results were not seen in the females of the F_1 generation (which were the same age). As the study author suggested - it is possible that the F_1 fish adapted or became more tolerant to the chemical and physical dynamics of the exposure system following their longer exposure duration (i.e. changes in physiological condition over time may have influenced VTG concentration). Moreover, adopting a cautionary approach, the NOEC and LOEC values for vitellogenin are considered to be 0.5 and 1.0 mg/L, respectively.

Table 75: The effect of cyproconazole on VTG concentration (expressed as mean concentration of VTG in individual fish) in male and female Fathead minnows of the F_0 and F_1 generations, as indicated.

Nominal Concentration (mg/L)	VTG Concentration (ng/mL)							
	Males				Females			
	n	F_0	n	F_1	n	F0	n	F_1
Water control	20	<LOD	27	<LOD	19	4583	13	80422
Solvent control	27	<LOD	25	<LOD	13	12709	15	82380
0.125	25	<LOD	22	<LOD	14	8909	18	45211
0.25	30	<LOD	22	<LOD	10	5380	18	116228
0.5	27	<LOD	22	<LOD	12	5052	18	46049
1.0	25	<LOD	27	<LOD	14	1104*	13	149711
2.0	21	<LOD	25	<LOD	19	<LOD*	15	87954

LOD = limit of detection.

* significant reduction in VTG (p = 0.05) compared to solvent control.

<u>Histopathology</u>:

Exposure of 92-93-day old fathead minnows to doses up to and including 2 mg/L cyproconazole (SAN 619) from 24hours post hatching onwards, did not result in any statistically significant increase in the combined numbers of fish with abnormal gonads (undifferentiated or ovo-testis) compared to control groups, in either the F_0 or F_1 generations. A male preponderance was present in all but the high dose (2 mg/L) F_0 test group (Table 76 below). However, there was no dose-response relationship between the dose of test substance and the male preponderance noted for either generation. At 2 mg/L, in the F_0 generation only, there was a minimal reduction in the number of male fish (18 in the treatment group versus 20 in the dilution water control).

However, this finding was not considered to be of toxicological significance and the NOEC and LOEC values for histopathological changes to the gonads of fathead minnows are considered to be 2.0 and > 2.0 mg cyproconazole/L, respectively.

Table 76: Sex ratios (as %) based on histopathological analysis of gonads in 92-93 day old Fathead minnows exposed to Cyproconazole.

Nominal Concentration (mg/L)	F_0 generation				F_1 generation			
	u/d* gonad	Male	Intersex (ovo-testes present)	Female	u/d* gonad	Male	Intersex (ovo-testes present)	Female
Water control	0	50	2.5	47.5	0	67.5	0	32.5
Solvent control	0	67.5	0	32.5	0	60	2.5	37.5
0.125	2.5	60	0	37.5	0	55	0	45
0.25	2.5	70	2.5	25	0	50	5	45

0.5	2.5	62.5	2.5	32.5	0	55	0	45
1.0	0	65	0	35	0	65	2.5	32.5
2.0	2.5	45	5	47.5	0	62.5	0	37.5

*u/d = undifferentiated gonad

A summary of the NOEC and LOEC values for the effects observed, are summarized in Table 77 below.

The lowest (most sensitive) biological parameter in the F_0 generation appeared to be egg production in females - reduced egg production was noted at nominal concentrations of 0.25 mg/L (giving an NOEC of 0.125 mg/L), when compared to dilution water controls. However, when the same dataset was compared to solvent controls, there was no significant difference between controls and fish exposed to test substance. As discussed earlier, the data showed high individual variability between individuals within a treatment and comparisons with historical control data indicated that both solvent type and solvent concentration can have quite a significant influence on egg production in fathead minnow. Therefore, interpretation of the egg-production data should be treated with caution as it may not be a reliable endpoint for risk assessment purposes. The most relevant endpoint in the F_0 generation was VTG concentration, which was significantly reduced in female fish when exposed to 1.0 mg ai/L and above (NOEC = 0.5 mg ai/L), though, similar results were not observed in the F_1 generation.

Egg hatchability appeared to be the most sensitive endpoint in the F_1 generation (NOEC and LOEC values were 0. 5 and > 0.5 mg ai/L, respectively), though these values are more likely a reflection of the poor egg production at 1.0 and 2.0 mg/L amongst this generation of fish, rather than effects on hatchability per se.

Table 77: **A summary of the relevant NOEC's and LOEC's for the effects of SAN 619F on various stages of the life cycle of Fathead minnows exposed to Cyproconazole.**

Biological parameter			Effect concentration [mg a.i./L]	
Stage	Day post hatch	Parameter	NOEC	LOEC
F_0 Generation				
F_0	-	Egg hatch	2.0[3]	>2.0[3]
F_0	-	Egg production	2.0[1]	>2.0[1]
F_0	-	**Egg production**	**0.125[2]**	**0.25[2]**
F_0	56	Survival	2.0[3]	>2.0[3]
F_0	92/93	Survival	1.0[3]	2.0[3]
F_0	92/93	Length	2.0[1,2,3]	>2.0[1,2,3]
F_0	92/93	Weight	2.0[1,2,3]	>2.0[1,2,3]
F_0	92/93	Vitellogenin (male)	2.0[1]	>2.0[1]
F_0	**92/93**	**Vitellogenin (female)**	**0.5[1]**	**1.0[1]**
F_0	92/93	Histopathology	2.0[1,2,3]	>2.0[1,2,3]

Biological parameter			Effect concentration [mg a.i./L]	
F[1] Generation				
F[1]		Egg hatch	0.5[3]	>0.5[3]
F[1] ELS 1	92/93	Survival	2.0[3]	>2.0[3]
F[1] ELS 1	92/93	Length	2.0[1,2,3]	>2.0[1,2,3]
F[1] ELS 1	92/93	Weight	2.0[1,2,3]	>2.0[1,2,3]
F[1] ELS 2	56	Survival	2.0[3]	>2.0[3]
F[1] ELS 2	56	Length	2.0[1,2,3]	>2.0[1,2,3]
F[1] ELS 2	56	Weight	2.0[1,2,3]	>2.0[1,2,3]
F[1]	92/93	Vitellogenin (male)	2.0[1]	>2.0[1]
F[1]	92/93	Vitellogenin (female)	2.0[1]	>2.0[1]
F[1]	92/93	Histopathology	2.0[1,2,3]	>2.0[1,2,3]

ELS = early life stage test No 1 or 2.

[1] value determined from a comparison between treatment groups and solvent control

[2] value determined from a comparison between treatment groups and dilution water control

[3] value determined from a comparison between treatment groups and combined dilution water and solvent controls

The most relevant 2-generation NOEC in fathead minnow exposed to cyproconazole for 357 days was considered to be 0.5 mg/L, based on endocrine effects (a decrease in VTG concentration) in female fish at 1.0 mg cyproconazole/L, relative to solvent controls. However, this was not the lowest (most sensitive) 2-generation NOEC. Effects on reproduction (reduced egg production in females) were noted at nominal concentrations of 0.25 mg/L (giving an NOEC of 0.125 mg/L), when compared to dilution water controls. However, if the same dataset were compared to solvent controls – there was no significant difference between controls and fish exposed to test substance. Therefore interpretation of the egg-production data should be treated with caution. The data showed high individual variability between individuals within a treatment, as indicated by the magnitude of the standard deviations and comparisons with historical control data indicates that both solvent type and solvent concentration can have quite a significant influence on egg production in fathead minnow. As a consequence of this variability, the statistical procedures are less powerful for detecting significant differences between treatments. Therefore egg production was not considered to be a reliable endpoint for risk assessment purposes.

In conclusion, the NOEC value of 0.50 mg a.i. /L is deemed the most appropriate value for chronic risk assessment in the fathead minnow as it is based on a more reliable biological parameter (VTG decrease), using more powerful statistical methods to assess differences between treatment groups and controls. It also incorporates all life stages of the fat head minnow (including a possible effect on egg hatchability in the F[1] generation).

5.4.2   Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Acute Toxicity of SAN 619 F To Daphnids (Daphnia magna); Suprenant D.C. November 1986:
The acute toxicity of SAN 619F was investigated by *Suprenant D.C. (1986)* using *Daphnia magna* as the species of choice in accordance with the *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-2 (October 1982)*. Four groups of 5 daphnids (*Daphnia magna*, neonates less than 24-hour old) were exposed to Cyproconazole at each of the six nominal test concentrations 7.8, 13, 22, 36, 60 and 100 mg /l (which corresponded to 19, 25, 27, 25, 14, 23 and

Concentration [mg a.i./L]		Mortality [%]		Exposure period [hours]	EC-$_{50}$*[mg a.i./L]
Nominal	Mean measured	24 h	48 h		
Water	-	0	0	24 h	> 22
Solvent	-	0	0	48 h	> 22
7.8	1.5	0	5	EC$_{100}$ = > 22 EC$_0$ = 1.5 48-h NOEC = ≤ 1.5 mg a.i./L	
13	3.3	0	15		
22	6.0	0	20		
36	8.9	0	30		
60	14	0	20		
100	22	6	35		

22% of the nominal concentration respectively), in a static test for 48 hours. Acetone was used as solvent (vehicle) to prepare the solutions (0.5 ml/L). Two additional groups were added as solvent control and water control. Temperature, pH, dissolved oxygen were recorded during the study. Concentrations of test solutions were analyzed at 0 hours. Mortalities, immobility and behavior were recorded at 24 and 48 hours.

Test temperatures were 2°C lower than the desired limits but this was not thought to affect the results of the study. Dissolved oxygen concentrations exceeded 60 % of saturation during the test period. The pH ranged from 7.8 to 8.4 over the course of the study**.**

Table 78:   **Effects of San 619 F on *Daphnia magna***

* calculated based from measured values

At 24 hours only the highest concentration of 100 mg/L (22%) resulted in mortalities of 6%, no mortalities were observed at the lower concentrations at 24 hours. At 48 hours the percentage mortalities ranged from 5 to 35% over the above six nominal test concentrations and resulted in a 48-hour EC$_{50}$ value greater than 22 mg a.i./L (only 35% immobilization) for cyproconazole in *Daphnia magna*, the highest concentration studied in this study. The NOEC was determined to be less than 1.5mg/L *Daphnia magna.*

Acute Toxicity of SAN 619 F to Daphnia magna; Frazier S. February 1988:

This acute invertebrate toxicity test was conducted in accordance with the *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-2 (October 1982).*

An initial range finding test with concentrations from 0.10 to 100 mg/L was conducted. From the results six treatment concentrations in duplicate, each containing ten *Daphnia magna* (first instar (<24-h old)) per beaker, were used in the definitive test. The nominal test concentrations were a logarithmic series ranging from 5.6 to 100 mg/L and included a control and a solvent (DMF,

0.01mg/mL) control. Samples were analysed for SAN 619F using a gas liquid chromatograph. Monitoring for immobility and abnormal behaviour was conducted at 4 hours and every 24 hours. Water temperature, dissolved oxygen and pH were monitored during the course of the study. Water temperature was constant at 20°C in all vessels at 0 and 48 hours. Dissolved oxygen ranged from 8.0 to 9.0 mg/L at 0 hours and from 8.3 to 8.4mg/L at 48 hours. The pH ranged from 8.0 to 8.2 at 0 hours and from 8.2 to 8.3 at 48 hours.

Table 79: **Effect of SAN 619F on *Daphnia magna* in acute toxicity study**

Concentration [mg ai/L]		Mortality [%]			Exposure period	EC_{50}* (95 % conf. interval)
Nominal	Mean measured	4 h	24 h	48 h	[hours]	[mg ai/L]
Water	-	0	0	0	4 h	62 (44-77)
Solvent	-	0	0	0	24 h	35 (31-41)
5.6	4.6	0	0	0	48 h	26 (22-32)
10	8.5	0	0	5	$EC_{100} = 77$ $EC_0 = 8.5$ **48-h NOEC** = 4.6 mg a.i./L	
18	15	0	0	20		
32	27	0	20	30		
56	44	0	75	90		
100	77	90	100	100		

* calculated based from measured values

The EC_{50} values at 4, 24, and 48 hours were 62, 35, and 26 mg/L respectively. All results were based on the mean measured concentrations of 4.6, 8.5, 15, 27, 44, and 77 mg/L (overall representing 82% of the nominal concentration). The NOEC was established as 4.6 mg/L. Abnormal effects of immobility and/or daphnids tending to the bottom of test vessels were observed at the other tested concentrations.

Cyproconazole: a 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia); Drottar K. and Swigert J.P. September 1993(a):

Saltwater mysids (<24 hours old) were exposed to a geometric series of five test concentrations of SAN 619F, a solvent control (DMF, 0.4 mL/L) and a negative control in accordance with the *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No.72-3(b) (October 1982), and ASTM Standard E 729-88 (1988) Guidelines*. The following nominal test concentrations were selected based on the results of a range finding test: 2.6, 4.3, 7.2, 12, and 20 mg SAN 619F/L. Mean concentrations were determined radio-chemically. Two replicate test chambers were maintained in each treatment and control group, with 10 mysids in each test chamber.

A continuous-flow diluter was used to deliver the test concentration and which was adjusted so that each test chamber received approximately 14 volume additions of test water every 24 hours. Photoperiod was set to 16/8 hours light/dark with a 30-minute transition period between light and dark. Temperature was measured continuously throughout the testing period with a target temperature of 25±1°C. The salinity of the water in the negative control was measured at the

beginning of the test. Dissolved oxygen and pH were measured in alternate test replicates of each treatment and control group at 24-hour intervals during the study.

The mean measured concentrations for the study were 2.8, 4.2, 7.1, 11, and 19 mg cyproconazole (SAN 619F) or 108, 98, 99, 92, and 95 % of nominal respectively. Temperatures were within the limits established for the test, 25±1°C. Dissolved oxygen concentration measurements exceeded 60 % of saturation throughout the test.

Daily observations of mortality and other signs of toxicity observed during the test period were recorded at 24, 48, 72 and 96 hours. Mysids in the negative control appeared normal throughout the testing period. The solvent control exhibited 5% mortality although the surviving mysids appeared normal. Mysids in the 2.8 mg SAN 619F/L treatment group appeared normal throughout the test with no mortalities or overt signs of toxicity. At the 96-hour mark 5 % mortality in the 4.2 mg/L treatment group, 25 % mortality of mysids in the 7.1 mg/L group, and 50 % mortality in the 11 mg/L treatment group were observed. Percentage mortality was 45 % within 72 hours and 100 % by 96 hours in the highest treatment group, 19 mg/L.

Table 80:   Effects of cyproconazole (SAN 619F) on mysids over a 96hr exposure period

Concentration [mg a.i./L]		Mortality [%]				Exposure period	EC-$_{50}$* (95 % conf. interval)
Nominal	Mean measured	24 h	48 h	72 h	96 h	[hours]	[mg a.i./L]
Water	-	0	0	0	0	24 h	> 19
Solvent	-	0	5	5	5	48 h	> 19
2.6	2.8	0	0	0	0	72 h	> 19
4.3	4.2	0	5	5	5	96 h	9.6 (8.6-11.3)
7.2	7.1	0	0	5	25	**EC$_{100}$** = 19 **EC$_{0}$** = 4.2 **48-h NOEC** = 4.2 mg a.i./L	
12	11	0	15	20	50		
20	19	10	25	45	100		

* calculated based on measured values

The 96-hour LC$_{50}$ value for saltwater mysids exposed to cyproconazole (SAN 619F) was determined to be 9.6 mg a.i./L. The no observed effect concentration (NOEC) was 2.8 mg a.i/L.

Cyproconazole (SAN 619F): A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica); Sved D.W. et al. September 1993:

The 96-hour acute toxicity of Eastern oysters, *Crassostrea virginica*, to cyproconazole was tested in accordance with the *U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision E, Section No.72-3(b) (October 1982)*. Eastern oysters were exposed to a geometric series of test concentrations based on the results of a range finding test. Nominal concentrations were 0.8, 1.3, 2.2, 3.6, and 6.0 mg SAN 619F/L and the mean measured concentrations were radio-chemically measured. A solvent control (DMF 0.3 mL/L) and a negative control were included in the experimental design. One test chamber was maintained for each treatment level each containing 20 oysters. Prior to adding the oysters to the test chambers all new shell growth was removed using a motorized grinder. The flow of unfiltered saltwater into each test chamber was approximately 1 L

water/oyster/hour. Algal cells (*Thalassiosira sp*.) were provided to supplement naturally occurring algae, and to maximise oyster growth rates. The salinity was measured in the negative control test chamber at the beginning, midpoint and end of the test.

Oysters were observed daily for mortality and clinical signs of toxicity. At the end of the test the longest finger of new shell growth on each oyster was measured to the nearest 0.05mm. The mean measured concentrations of cyproconazole (SAN 619F) were 0.66, 1.2, 1.9, 3.0, and 5.4 mg/L which corresponded to 83, 92, 86, 83, and 90 % of nominal, respectively. Temperatures were within the 22±1°C limits established for the test.

No mortalities were observed during the test. Oyster shell growth in the negative control averaged approximately 4.4 mm over the 96-hour test period, while oyster shell growth in the solvent control group averaged 3.5 mm, a difference which was statistically significant. Therefore, the measurements of the solvent control group were used to analyse the growth inhibition in the treatment groups. Oyster shell growth in the 0.66, 1.2, 1.9, 3.0, and 5.4 mg/L treatment groups averaged 3.5, 3.7, 3.9, 0.7, and 0.0 mm, respectively. Statistically significant shell growth inhibition was observed in the 3.0 and 5.4 mg/L treatment groups.

Table 81:   The effect of cyproconazole (SAN 619F) on shell growth in Eastern oyster

Concentration [mg a.i./L]		Mortality after 96 h	Shell growth** inhibition after 96 h	Exposure period	LC-$_{50}$* (95 % conf. interval)
Nominal	**Mean measured**	**[%]**	**[%]**	**[hours]**	**[mg a.i./L]**
Seawater	0	0	(-25)	96-h	2.6 (1.9-3.0)
Solvent	0	0	-		
0.8	0.66	0	0.3		
1.3	1.2	0	0		
2.2	1.9	0	0	**96-h NOEC:** 1.9 mg ai/L	
3.6	3.0	0	79.7a		
6.0	5.4	0	100a		

[a] statistically significantly different from the control
* calculated based on nominal values   ** compared to the solvent control

The 96-hour EC$_{50}$ of cyproconazole (SAN 619F) to eastern oysters was determined to be 2.6 mg a.i./L, with a NOEC of 1.9 mg a.i./L.

Cyproconazole is of toxic to aquatic invertebrates based on the definitive EC$_{50}$ values determined from the key study and supportive study described above.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Cyproconazole (SAN 619 F): a flow-through life-cycle toxicity test with the Cladoceran (Daphnia magna), Drottar, K.R and Swigert, J.P. October 1993(c):

The effects of SAN619F (cyproconazole) on the life-cycle of *Cladoceran (Daphnia magna)* was investigated in accordance with the *U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Section No. 72-4.*

Daphnids were exposed to a geometric series of five test concentrations of SAN619F, a solvent (DMF0.05mL/L) control, and a negative (well water) control. Nominal test concentrations were

0.15, 0.30, 0.60, 1.2, and 2.4 mg cyproconazole (SAN 619F)/L based on the results of a range-finding test. Mean concentrations were determined radio-chemically at test initiation, Days 7 and 14, and at test termination. Two replicate test chambers were maintained in each treatment and control group. Each treatment group consisted of eight test compartments containing one daphnid and four test compartments containing five daphnids. Daphnid neonates less than 24-hours old were used in the test. Observations of mortality, reproduction, and other clinical signs were made at least three times a week. The no observed effect concentration (NOEC) was determined by examination of the mortality, growth, and reproduction data.

Mean measured concentrations of the test substance were 0.29, 0.57, 1.1, and 2.3 mg cyproconazole (SAN 619F)/L, respectively which represented a range from 94 to 96 % of nominal. After 21 days of exposure, survival was $\geq$ 89 % in all treatment and control groups. Statistically, survival was not significantly reduced in any treatment group in comparison to the pooled controls. At test termination, all surviving daphnids appeared normal. The no observed effect concentration for survival was 2.3 mg cyproconazole (SAN 619F)/L.

Treatment-related reductions in reproduction were observed in the 0.57, 1.1, and 2.3 mg cyproconazole (SAN 619F)/L. Consequently, the lowest observed effect concentration was 0.57 mg cyproconazole (SAN 619F)/L and the no observed effect concentration was 0.29 mg cyproconazole (SAN 619F)/L. Reproduction was the most sensitive parameter measured in the chronic toxicity test.

Differences in carapace length did not appear to be concentration dependent and were not statistically significant using analysis of variance. However, a concentration dependent reduction in weight was observed in the 1.1 and 2.3 mg cyproconazole (SAN 619F)/L treatment groups. Consequently, the lowest observed effect concentration for growth was 1.1 mg cyproconazole (SAN 619F)/L and the NOEC was 0.57 mg cyproconazole (SAN 619F)/L.

Table 82:   Effects on the parental generation (results from 4 replicates with 5 daphnids)

Concentration [mg a.i./L]		Immobilisation / Mortality [%]				Exposure period	EC-$_{50}$* (95 % conf. interval)
Nominal	Mean measured	3 d	8 d	15 d	21 d	[hours]	[mg a.i./L]
Water	-	0 / 0	0 / 0	0 / 4	0 / 4	2 d	> 2.3
Solvent	-	0 / 0	0 / 0	0 / 0	0 / 0	7 d	> 2.3
0.15	0.14	0 / 0	0 / 0	0 / 0	0 / 0	14 d	> 2.3
0.30	0.29	0 / 0	0 / 4	0 / 11	0 / 11	21 d	> 2.3
0.60	0.57	0 / 0	0 / 0	0 / 11	0 / 11		
1.2	1.1	0 / 0	0 / 0	0 / 0	0 / 0	**EC$_{100}$ = > 2.3**	
2.4	2.3	0 / 0	0 / 0	0 / 0	0 / 0	**21-d NOEC = $\geq$ 2.3 mg a.i./L**	

* calculated based from measured values

Table 83:   Effects on reproduction (results from 8 replicates with 1 Daphnid)

Concentration [mg a.i./L]		Mean Cumulative Nb of Youngs produced (day 21)	Length	Dry weight	Exposure period	EC-$_{50}$* (95 % conf. interval)
Nominal	Mean measured	[Nb]	[mm ±SD]	[mg ±SD]	[hours]	[mg a.i./L]
Water	-	122	4.8±0.29	1.11±0.21		
Solvent	-	120	4.8±0.26	1.04±0.23		
0.15	0.14	123	5.0±0.51	1.11±0.22	21 d	0.74
0.30	0.29	99	4.8±0.24	1.05±0.17		
0.60	0.57	91**	4.6±0.26	1.09±0.13		
1.2	1.1	32**	4.5±0.59	0.78±0.17[**]	**21-d NOEC** = 0.29 mg a.i./L	
2.4	2.3	33**	5.0±0.54	0.81±0.09[**]		

* calculated based from measured values

** result significantly different from controls (Bonferroni's T-test , P ≤ 0.05)

The mean number of young produced on day 21 in the controls was 122 confirming validity criteria f or the reproduction study. The 21 day no observed effect concentration (NOEC) for the effects on the parental generation in *Daphnia magna* was >2.3 mg cyproconazole/L (the highest concentration tested) and the NOEC for reproduction was 0.29 mg cyproconazole/L.

Cyproconazole (SAN 619) technical: Effect on survival, growth and reproduction of Daphnia magna in a semi-static test over three weeks, Bätscher, R. October 2006:

The toxicity of cyproconazole (SAN619) technical on the survival, growth (body length) and reproduction of *Daphnia magna* was determined in accordance with OECD Guideline 211 – *Daphnia magna* Reproduction test.

In this semi-static test Daphnids were exposed to a geometric series of five test concentrations of cyproconazole technical and a negative (dilution water) control. Nominal test concentrations were 0.023, 0.073, 0.23, 0.73, and 2.3 mg cyproconazole technical/L. The concentrations of cyproconazole in the test solutions from the beginning and end of three test medium renewal periods were measured using LC analysis with MS-detection. Two renewal periods of 48 hours and one renewal period of 72 hours were chosen for sampling. The analytical measurements were performed in the test media of the lowest and the highest nominal test concentrations of 0.023 and 2.3 mg/L.

Each treatment group consisted of 10 Daphnids per treatment (1 Daphnid per replicate) with each invertebrate individually maintained in a 100 mL glass beaker containing 80 mL of test medium. The test media of all test concentrations and of the control were renewed every two to three days. At the beginning and end of each test medium renewal period, the pH values, dissolved oxygen concentrations, the water temperature and appearance of the test media were recorded. At the renewals of the test media, the surviving test specimens were carefully transferred with the aid of glass tubes from the old test vessels into the freshly prepared test media of the corresponding concentrations. The test specimens were fed on each working day with a food mixture containing

one part of green algae of the species *Scenedesmus subspicatus* and one part of fish food suspension.

Observations of mortality, reproduction, and other clinical signs were made and the no observed effect concentration (NOEC) was determined by examination of the mortality, growth, and reproduction data. The reproduction rate was calculated as the total number of living offspring produced per parent female surviving until the end of the test (Table 84). The body length of the adults was measured at the end of the test by measuring the daphnids from the top of the head to the base of the spine using a binocular microscope.

The NOEC and the LOEC were statistically evaluated by testing the mean reproduction rate and the mean body length at the test concentrations for statistically significant differences to the control by multiple Williams-tests. The highest concentration of cyproconazole technical tested without toxic effects after the exposure period of 21 days (21-day NOEC) was 0.023 mg/L. The lowest concentration tested with toxic effects (21-day LOEC) was determined to be 0.073 mg/L due to the statistically significantly reduced mean reproduction rate of *Daphnia magna* at this test concentration.

The EC$_{50}$ for the inhibition of the reproduction rate after 21 days was calculated by Moving Average Interpolation. The EC$_{50}$ was determined to be 0.83 mg/L (95% confidence limits could not be determined).

Table 84: **Influence of cyproconazole (SAN619) technical on *Daphnia magna* following exposure for 21 days in a semi-static test**

	Control	Cyproconazole (SAN619) technical (nominal concentration in mg/L)				
		0.023	0.073	0.23	0.73	2.3
Mortality after 21 days of exposure (%)	10	0	10	20	0	10
Mean reproduction rate (living offspring per surviving adult)	77.4	69.8	57.2*	50.6*	57.7*	0.0*
Mean reproduction rate in % of control	-	90.1	73.9	65.4	74.5	0.0
Mean body length of the adults (mm)	3.81	3.71	3.74	3.79	3.78	3.77
Mean body length of the adults as % of control	-	97.4	98.2	99.5	99.1	99.0

* statistically significantly lower than the control value (results of a Williams-test, one-sided smaller, $\alpha = 0.05$

The highest concentration of cyproconazole technical tested without toxic effects to *Daphnia magna* after the exposure period of 21 days (21-day NOEC) was 0.023 mg/L. The lowest concentration tested with toxic effects (21-day LOEC) was determined to be 0.073 mg/L due to the statistically significantly reduced mean reproduction rate at this test concentration.

5.4.3   Algae and aquatic plants

5.4.3.1 Short-term toxicity to algae and aquatic plants

Acute toxicity of SAN 619F to Scenedesmus subspicatus (OECD: Algae Growth Inhibition Test); Ellgehausen H. November 1986(a):

The investigation of the acute toxicity of SAN 619F (cyproconazole) to *Scenedesmus subspicatus* was undertaken in accordance with the *OECD Guideline for the testing of chemicals: Freshwater Algae and Cyanobacteria, Growth Inhibition Test (OECD 201, 1984).*

Scenedesmus subspicatus was cultured in 0.125, 0.25, 0.5, 1.0 mg/L SAN 619F for 96 hours. The experimental design included an untreated control and potassium dichromate which was the reference compound. All tests were run in triplicate under continuous illumination with initial cell volumes of 10^4 cells/ml. Incubation flasks were 50ml Erlenmeyer flasks stoppered with cotton wool plugs and the test run for 96 hours. Algae samples were taken after 24, 48, 72, and 96 hours of incubation and the number of algae spectro-photometrically determined. The percentage inhibition of algal growth was determined for 72 and 96 hours of incubation.

The EC_0 and EC_{100} of cyproconazole to *Scenedesmus subspicatus* was determined to be 0.021 mg/L and 5.8 mg/L respectively. The EC_{50} was determined by Logit analysis and was 0.077 mg/L. The EC_{50} of potassium dichromate was 0.998 mg/L.   Concentrations of cyproconazole in the test solutions were analyzed by gas chromatography. The recoveries were 75.2, 90.2 and 93.9% of the nominal values of 0.125, 0.5 and 1.0 mg/L, respectively. These results gave a mean % of recovery of 86.43% and this value was used to estimate the actual concentrations of cyproconazole in the test solutions at nominally 0.032, 0.063 and 0.25 mg a.i./L. Effects were based on measured concentrations.

Table 85:   Effects of cyproconazole (SAN 619F) on *Scenedesmus subspicatus*

Concentration [mg a.i./L]		Mean No. of algal cells per ml (x10^4)				Inhibition [%]		Exposure period	EC-50* (95 % conf. interval)
Nominal	Measured[1]	24h	48h	72h	96h	72h	96h	[hours]	[mg a.i./L]
Water	-	10.0	30.6	133.5	245.1	-	-	24 h	> 1
0.032	0.028[2]	10.8	25.9	98.4	232.9	20.5	15.2	48 h	> 1
0.063	0.055[2]	9.9	19.9	48.8	144.2	50.7	49.2	72 h	0.099
0.125	0.094	11.1	16.6	26.5	46.9	63.3	73.8	96 h	0.077 (0.075-0.08)
0.25	0.216[2]	11.9	14.9	20.7	29.2	66.9	79.1		
0.5	0.451	8.8	10.5	14.4	21.0	77.1	85.1	**ECb100** = 5.8	
1.0	0.939	9.1	10.5	12.7	19.3	77.6	85.9	**96-h NOEC** = 0.021 mg a.i./L	

[1] mean of two measured values
[2] calculated assuming measured values are 86.43% of nominal

The EC_0 of SAN 619 F was established as 0.021 mg/L and the EC_{100} was 5.8 mg SAN 619 F/L. The 96 –hour E_bC_{50} was determined to be 0.077 mg SAN 619F/L and the 72-hour E_bC_{50} was determined to be 0.099 mg SAN 619F/L

SAN 619F: Determination of EC₅₀ to Chlorella vulgaris (72-hour static assay); Jenkins W.R. November 1993:

The investigation of the acute toxicity of SAN 619F (cyproconazole) to _Chlorella vulgaris_ was undertaken in accordance with the _OECD Guideline for the testing of chemicals: Freshwater Algae and Cyanobacteria, Growth Inhibition Test (OECD 201, 1984)._

Replicate algal cultures with an initial cell count of 1×10^4/ml were exposed to SAN 619F in mineral salts medium at nominal concentrations of 50, 100, 200, 400, 800, and 1600 µg/L or to mineral salts alone. The test concentrations were chosen based on the results of a range-finding test. GC analysis of the media indicated that concentrations of SAN 619F were adequately maintained (between 90 and 103 % of their nominal concentrations) giving overall mean measured concentrations of 0.045.9, 0.097.5, 0.196, 0.392, 0.820, and 1.530 mg/L. Nine flasks were established for each exposure group and ten were established for the controls. Incubation temperature was at 22-24°C and illumination in the incubation chamber was constant. The cell densities of three test cultures at each concentration and ten control cultures were measured using a haemocytometer, at 24-hour intervals for 72 hours. Growth rate and biomass were both calculated.

Exposure of _Chlorella vulgaris_ to SAN 619F at measured levels of 0.820 mg/L and above resulted in a significant reduction in average specific growth rate and biomass compared to control cultures. Biomass was significantly greater than the control at 0.196 mg/L but this was not believed to be treatment related. Thus, the no observed effect concentration (NOEC) for growth rate and biomass was 0.392 mg/l The 50 % effect concentrations for average specific growth rate (E_rC_{50}) and mean biomass (E_bC_{50}) respectively were found to be 1.176 mg/L and 0.660 mg/L.

At the end of the test, in order to establish whether toxic levels of SAN 619F caused inhibition of algal growth (i.e. algistatic) or algal cell death (i.e. algicidal), samples from cultures at the highest exposure level (1.530 mg/L) were diluted (1:100) with fresh culture medium. Following incubation for five days, these subcultures showed normal growth, indicating that at 1.530 mg/L the test material was algistatic.

Table 86:   Effects of SAN 619 F on _Chlorella vulgaris_ (0-72 hours)

Concentration [mg a.i./L]		Mean No. algal cells per ml (x10⁴)			Inhibition at 72h [%]		Exposure period [hours]	EC-₅₀* (95 % conf. interval) [mg a.i./L]
Nominal	Actual	24h	48h	72h	AUC	Growth		
Water	-	5.0	37.7	117.3	-	-	72 h biomass 72 h growth	0.66 (0.609-0.718) 1.176 (1.03-1.37)
0.05	0.0459	4.1	45.2	140.4	-18.6	-3.77		
0.1	0.0975	4.5	46.8	126.4	-13.5	-1.57		
0.2	0.196	4.0	51.8	143.6	-26.8	-4.25		
0.4	0.392	2.1	35.1	115.0	6.5	0.42		
0.8	0.82	2.5[a]	9.0[a]	26.9[a]	77.2a	30.91[a]	**72-h NOEC** = 0.392 mg a.i./L	
1.6	1.53	2.3[a]	2.7[a]	5.8[a]	94.5[a]	63.11[a]		

[a] statistically different from the control
* calculated based from measured values
AUC area under the curve

The 72-hour EC_{50} of cyproconazole in the green algae *Chlorella vulgaris* was $E_rC_{50} = 1.176$ mg/L and $E_bC_{50} = 0.66$ mg/L. The recovery test showed that effects of cyproconazole are reversible and temporary on algal growth at least up to a concentration of 1.53 mg ai/L, the highest concentration tested. This indicates that cyproconazole at concentrations up to at least 1.53 mg a.i./L are not algicidal but algistatic.

Cyproconazole is of high toxicity to algae based on the definitive E_bC_{50} value determined from both the key study and supportive study.

5.4.3.2 Long-term toxicity to algae and aquatic plants

Not relevant for this dossier.

5.4.4   Other aquatic organisms (including sediment)

5.4.4.1 Short-term toxicity to other aquatic organisms (including sediment)

SAN619 (Cyproconazole technical): Effect on the Respiration Rate of Activated Sludge; Wallace S.J. June 2002:

The acute toxicity of Cyproconazole through the inhibition of activated sewage sludge respiration was investigated by Wallace, S.J. (2002) using the *OECD Guideline for the testing of chemicals: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation).*

The effect of cyproconazole on bacterial activity of sewage sludge was investigated by exposing activated sludge to cyproconazole at nominal concentrations of 1, 3.2, 10, 32 and 100 mg/L. The test incorporated a dose response to a reference standard (3,5-dichlorophenol) at five nominal concentrations of 1, 3.2, 10, 32 and 100, and 2 replicates of an untreated control. After continuous aeration for 3 hours at 20±2°C, the respiration rate of each culture was determined by measuring oxygen consumption. The respiration rates of treated cultures were expressed as a percentage of the mean rates seen in control cultures.

Inhibition values below 10% are within expected experimental variability and are not considered to be a consequence of exposure to the test substance. 3,5-DCP caused significant reductions in the rate of oxygen consumption thus confirming that the activated sludge was responding normally and contained viable sludge organisms.

Table 87:   Effect of cyproconazole on oxygen consumption of activated sludge

Test substance	Concentration (mg/L)	O_2 consumption rate (mg/L/h)	Percent inhibition (%)
None	0	29.3	-
	0	31.7	-
Cyproconazole	1.0	29.3	<10
	3.2	28.5	<10
	10	27.4	10
	32	29.5	<10
	100	26.0	15

Test substance	Concentration (mg/L)	O₂ consumption rate (mg/L/h)	Percent inhibition (%)
3,5-DCP	3.2	27.4	10
	10.0	13.7	55
	32.0	6.0	80
	100	2.7	91

The inhibitory effects of Cyproconazole and the reference chemical 3,5-dicholorphenol (potent respiration inhibitor) on activated sludge respiration were compared to 3,5-DCP and demonstrated that 3,5-DCP induced a significant reduction in respiration (91% inhibition) at 100 mg/L compared to cyproconazole which induced 15% respiration inhibition at the same concentration. From these results it can be interpreted that cyproconazole is not significantly inhibitory at concentrations at or below 100 mg a.i./L.

5.4.4.2 Long-term toxicity to other aquatic organisms (including sediment)

Toxicity test of SAN 619 tech. on sediment-dwelling Chironomus riparius (syn. Chironomus thummi) under static conditions; Grade R. April 1999:

Exposure scenario A: Water phase; The test was performed by applying a range of concentrations of SAN 619 to the water column of sediment-water systems containing 20 first instar 2-3 day old larvae of *Chironomus riparius*, each under static conditions. The concentrations, based on a range-finding test were: 0.63, 1.25, 2.5, 5.0, 10 and 20 mg a.i. /L. Twenty four hours (24-hours) after the addition of the test organisms, the test substance was introduced by pipetting below the surface into the water column of the test system simulating a spray drift exposure scenario.

Exposure scenario B: Sediment phase: SAN 619 treated sand was mixed with artificial sediment at a range of concentrations based on the results of a range finding test: 12.5,25, 50, 100, 200 mg/kg sediment (dry weight). The Spiked sediment and water were added to the test vessels approximately 48 hours prior to the introduction of *Chironomus* larvae, simulating a run-off event.

The tests were performed at a constant temperature of 20 ± 2°C with a photoperiod of 16/8 light /dark with a 30 minute transition period. The biological assessment was based on impacts on full maturation of the larvae to adult midge over 28 day exposure. The main parameters examined were the rate and time of emergence and the total number of fully emerged male and female midges.

Exposure scenario A: The actual measured concentrations of SAN 619 in the water phase were 0.4, 0.8, 1.6, 2.6, 5.8, and 11.8 mg/L at day 0. At test termination these had decreased to 0.2, 0.4, 0.9, 1.8, 4.5, and 9.3 mg/L. Test substance concentrations in sediment were analysed in the 20 and 10 mg/L test concentrations. At day 0, 7 and 28 the measured test substance concentrations in the sediment (incl. interstitial water) were 8.75, 10.08 and 16.0 mg a.i./kg sediment (wet) at a nominal concentration of 10 mg a.i./L. At the nominal concentration of 20 mg/L the determined concentrations of cyproconazole were 18.5, 18.4 and 30.1 mg a.i./kg sediment (wet) at day 0, 7 and 28, respectively. Total recovery from test system at the nominal concentration of 10 mg test substance/l was 77, 88 and 80 % of the nominal concentration at day 0, 7 and 28. The corresponding figures for the nominal concentration of 20 mg/L were equivalent to 79, 83 and 79 % of the nominal concentration at day 0, 7 and 28. Therefore, in both cases, the overall mean was > 80%.

Table 88:   **Results of exposure of *Chironomids* to SAN 619 in exposure scenario A**

Concentration Nominal [mg a.i./L]	Emergence Rate [Mean]	Development Rate [Mean]	28-d $E_{EM}C_{50}$* [mg a.i./L] 10.6	28-d $E_{DE}C_{50}$* [mg a.i./L] 16.6
0	0.90	0.06875		
0.63	1.00	0.06641	28-d $E_{EM}C_{10}$* [mg ai/L] 9.8	28-d $E_{DE}C_{10}$* [mg ai/L] 9.0
1.25	0.97	0.06827		
2.5	1.00	0.06788		
5.0	1.00	0.06697	28-d $NOEC_{EM}$ [mg ai/L] 10	28-d $NOEC_{DE}$ [mg ai/L] 5
10	0.82	0.05838		
20	0.00	0.00		

* $E_{EM}C_{50}$: Endpoint (EC$_{50}$) Emergence rate ; $E_{DE}C_{50}$: Endpoint (EC$_{50}$) Development rate

Exposure scenario B: The actual measured test concentrations of SAN 619 in the sediment phase were: 0.2, 0.4, 0.9, 2.0, and 4.1 mg/L at day 0. At test termination the concentrations were: 0.4, 1.5, 2.7, 7.4, and 15.2 mg/L. Test substance concentrations in sediment were analyzed from samples with the highest administration rate, 200 mg a.i./kg, and the 100 mg a.i./kg rate. At days 0, 7 and 28 the concentrations in the sediment (incl. interstitial water) were 72.0, 70.0 and 57.9 mg a.i./kg wet sediment at the nominal concentration of 100 mg a.i./kg dry sediment (equivalent to 74.4 mg a.i./kg wet sediment). At the nominal concentration of 200 mg a.i./kg dry sediment (equivalent to 148.7 mg a.i./kg wet sediment), the concentrations of cyproconazole determined on days 0, 7 and 28 were 152.2, 136.8 and, 119.1 mg a.i./kg wet sediment, respectively.

Total recovery (water and sediment) from test system at the nominal concentration of 100 mg a.i./kg was 106, 118 and 111 % of the nominal concentration at day 0, 7 and 28. The corresponding figures for the nominal concentration of 200 mg a.i./kg were 112, 115 and 115 % of the nominal concentration at day 0, 7 and 28.

There were no indications of a difference in sensitivity to cyproconazole between the sexes; therefore male and female results were pooled for statistical analyses. Calculations of effect concentrations for the rate of emergence, the development time and the rate of development (reciprocal of the development time) were based on nominal concentrations in the spiked sediment.

Table 89:   Results of exposure of *Chironomids* to SAN 619 in exposure scenario B

Concentration [mg a.i./kg dry weight] Nominal	Emergence Rate [Mean]	Development Rate [Mean]	28-d $E_{EM}C_{50}$* [mg a.i./kg sediment] 88.0	28-d $E_{DE}C_{50}$* [mg a.i./kg sediment] 10386
0	0.85	0.06137	28-d $E_{EM}C_{10}$* [mg a.i./kg sediment] 66	28-d $E_{DE}C_{10}$* [mg a.i./kg sediment] 155
12.5	0.85	0.06195		
25	0.78	0.06110		
50	0.83	0.05930	28-d $NOEC_{EM}$ [mg a.i./kg	28-d $NOEC_{DE}$ [mg a.i./kg
100	0.22	0.05453		

200	0.017	0.02020	sediment] 50	sediment] 50

* $E_{EM}C_{50}$: Endpoint (EC_{50}) Emergence rate ; $E_{DE}C_{50}$: Endpoint (EC_{50}) Development rate

The EC_{50} values for emergence rate and development rate of *Chironomus riparius* were 10.6 and 16.6 mg a.i./L for organisms exposed to cyproconazole via spiking of the water column. The corresponding NOEC values were 10.0 and 5.0 mg a.i./L for emergence rate and development rate, respectively

The EC_{50} values for emergence rate and development rate of *Chironomus riparius* were 88.0 and 10386 mg a.i./kg dry sediment for organisms exposed to cyproconazole via spiking of the sediment. The corresponding NOEC values were 50 mg a.i./kg for both emergence rate and development rate.

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

The toxicity profile of Cyproconazole was generally consistent and is considered of sufficient quality to characterize the parent compound in terms of its hazard classification. The acute toxicity of Cyproconazole was investigated in fish, invertebrates (including daphnia, saltwater mysid and the eastern oyster), algae and aquatic microbials. The critical endpoint for acute toxicity was from the study conducted by *Ellgehausen, H. (1986a)* on *Scenedesmus subspicatus*. The 96 hr E_bC_{50} from this study was 0.077 mg a.i./L.

The chronic toxicity of Cyproconazole was investigated in fish, invertebrates and sediment dwelling organisms. The critical endpoint for chronic toxicity again used from the study conducted by *Ellgehausen, H. (1986a)* on *Scenedesmus subspicatus*. The NOEC from this study was 0.021 mg a.i./L These endpoints are used for the classification and labeling of Cyproconazole.

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

As Cyproconazole, although not readily bio-degradable, has a low bioaccumulation potential in fish and other aquatic organisms and therefore classification is as follows: In accordance with the classification criteria of the CLP Regulation (*EC 1272/2008 and the Guidance to Regulation (EC) No. 1272/2008 on Classification, Labelling and Packaging of substances and mixtures*) Cyproconazole is assigned a hazard statement Env. Aquatic Acute Category 1-H400: very toxic to aquatic life and Env. Aquatic Chronic Category 1-410: very toxic to aquatic life with long lasting effects.

As Cyproconazole is classified as category Acute 1 and category Chronic 1 a multiplying factor (M-factor) must be assigned in accordance with Article 10 of the CLP Regulation (as per Table 4.1.3 Annex 1 to CLP). For acute toxicity an M-factor of 10 is applied for cyproconazole. For chronic toxicity an M-factor of 10 is applied for cyproconazole. The Signal Word 'Warning' and the environmental hazard pictogram (GHS09) are required. The Precautionary Statements are: P273, P391, P501.

6   OTHER INFORMATION

No data.

7 REFERENCES

7.1 Physical and chemical properties

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Atkins, E.L.	1986	SAN 619F : Acute contact LD50 - Honey bee Novartis Crop Protection AG, Basel, Switzerland University of California, Riverside, United States, Report No N/A Not GLP Not Published Syngenta File N° SAN619/5952
Baetscher R.	2006	Cyproconazole (SAN619) technical - Effect on survival, growth and reproduction of Daphnia magna in a semi-static test over three weeks Syngenta Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, A99191 T022815-04 GLP, not published Syngenta File No SAN619/8244
Barth, M	2001	Cyproconazole: Toxicity of the formulation A-9898 A on the reproduction of the Collembola Folsomia candida Syngenta Crop Protection AG, Basel, Switzerland BioChem agrar, Gerichshain, Germany, Report No 01 10 48 040 GLP Not Published Syngenta File N° SAN619/7115
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Beavers, J.B.	1985c	SAN 619F - Dietary LC50 with the Bobwhite Quail Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., St. Michaels, United States, Report No 102-117 GLP Not Published Syngenta File N° SAN619/5958
Beavers, J.B., et, al.	1991	SAN 619 F : A dietary LC50 study with the Northern Bobwhite Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 131-153A GLP Not Published Syngenta File N° SAN619/5395
Beavers, J.B., et, al.	1993a	Cyproconazole: a reproduction study with the mallard Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 131-167 GLP Not Published Syngenta File N° SAN619/5041
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Beech, P.	1994	A Laboratory Evaluation of the Side-Effects of the Fungicide ALTO 100 SL on Adults of the Carabid Beetle Poecilus Cupreus Novartis Crop Protection AG, Basel, Switzerland Report No SAN-94-4 GLP Not Published Syngenta File N° SAN619/6684

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Blumhorst, M.R.	1995	Anaerobic aquatic metabolism of ^{14}C-Cyproconazole Novartis Crop Protection AG, Basel, Switzerland Epl Bio-Analytical Services Inc., Harristown, United States, Report No 111S16 GLP Not Published Syngenta File N° SAN619/6410
Bowman, J.H.	1988a	Acute Toxicity of SAN 619 F Technical to Rainbow Trout (Salmo gairdneri). Novartis Crop Protection AG, Basel, Switzerland ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States, Report No 36546 GLP Not Published Syngenta File N° SAN619/5965
Bowman, J.H.	1988b	Acute Toxicity of SAN 619 F Technical to Bluegill Sunfish (Lepomis macrochirus) Novartis Crop Protection AG, Basel, Switzerland ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States, Report No 36545 GLP Not Published Syngenta File N° SAN619/5950
Cafarella M	2009	Cyproconazole - Fish Full Life-Cycle Test with Fathead Minnow (Pimephales promelas) Syngenta - Jealott's Hill, Bracknell, United Kingdom Springborn Laboratories Inc., Wareham, USA, 1781.6681 , T005205-06 GLP, not published Syngenta File No SAN619_10010
Cafarella M	2008	Cyproconazole Technical Short-Term Reproductive Assay with Fathead Minnow (Pimephales promelas) Syngenta - Jealott's Hill, Bracknell, United Kingdom Springborn Laboratories Inc., Wareham, USA, 1781.6674, T001285-07 GLP, not published Syngenta File No SAN619_10003
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Caunter, J.E., Williams, T.D.	2001	Cyproconazole tech. (SAN 619): Determination of effects on the life cycle of the fathead minnow (Pimephales promelas), including measurements of vitellogenin and gonad histopathology Syngenta Crop Protection AG, Basel, Switzerland Brixham Environmental Laboratory, Brixham, United Kingdom, Report No BL7106/B GLP Not Published Syngenta File N° SAN619/7100
Donat, H.J.	1985	Laboratory studies on the acute contact and oral toxicities of SAN 619 F (active ingredient) to worker honeybees Novartis Crop Protection AG, Basel, Switzerland Sandoz AG, Basel, Switzerland, Report No PB NO. 66562/85a Not GLP Not Published Syngenta File N° SAN619/6426
Dorgerloh, M. and Sommer H.	2002	1,2,4-Triazole: Juvenile Growth Test on Fish (Oncorhychus mykiss) Syngenta Crop Protection AG, Basel, Switzerland Bayer AG, Leverkusen, Germany, Report No DOM21060 GLP Not Published Syngenta File N° CGA71019/0052
Drottar, K.R., et, al.	1993	Cyproconazole: a 96-hour flow-through acute toxicity test with the sheepshead minnow (Cyprinodon Variegatus) Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 131A-151A GLP Not Published Syngenta File N° SAN619/5047
Drottar, K.R., Swigert, J.P.	1993a	Cyproconazole (SAN 619): an early life-stage toxicity test with the Rainbow trout (Oncorhynchus mykiss) Novartis Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton, MD, United States, Report No 131A-153 GLP Not Published Syngenta File N° SAN619/5039
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Ehlers, H.A.	2000	Effects of 1,2,4-triazole on reproduction and growth of Earthworms Eisenia fetida (Savigny 1826) in artificial soil Novartis Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 7781022 GLP Not Published Syngenta File N° CGA64250/4385
Ellgehausen, H.	1986a	Acute toxicity of SAN 619 to Scenedesmus subspicatus Novartis Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No 075521 GLP Not Published Syngenta File N° SAN619/0104
Ellgehausen, H.	1986b	SAN 619 F - Acute Toxicity (LC50) Study in Earthworms (Eisenia foetida) Novartis Crop Protection AG, Basel, Switzerland RCC Ltd., Itingen, Switzerland, Report No RCC 075532 GLP Not Published Syngenta File N° SAN619/5954
Everett C., Wyeth K., Powley W.	2007	Cyproconazole (SAN619) - Growth Inhibition to Lemna gibba under Semi-static Conditions Syngenta Crop Protection AG, Basel, Switzerland Syngenta - Jealott's Hill International, Bracknell, Berkshire, United Kingdom, T002449-06-REG GLP, not published Syngenta File No SAN619/8293
Forbis, A.D.	1986	Uptake, depuration and bioconcentration of 14C-SAN 619 F to Bluegill Sunfish (Lepomis Macrochirus) Novartis Crop Protection AG, Basel, Switzerland ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States, Report No 35080 GLP Not Published Syngenta File N° SAN619/0109
Frazier, S.	1988	Acute Toxicity of SAN 619 F to Daphnia magna. Novartis Crop Protection AG, Basel, Switzerland ABC Analytical Bio-Chemistry Lab. Inc., Columbia, United States, Report No 36547 GLP Not Published Syngenta File N° SAN619/5938
Frey L., Martin K., Beavers J., Jaber M.	2007	Cyproconazole - A reproduction study with the Mallard Syngenta Crop Protection AG, Basel, Switzerland Wildlife International Ltd., Easton MD, USA, 528-238 T002365-03 GLP, not published Syngenta File No SAN619/8515
Glänzel A.	1999	Hydrolysis of ^{14}C-triazole labelled SAN 619 F under laboratory conditions. Novartis Crop Protection AG, Basel, Switzerland. GLP: Yes Not published Study number 99AG04 (Syngenta N° SAN619/6849)

Author(s)	Year	Title Source Other (GLP status; Published or not; Report no.)
Grade, R.	1999	Toxicity test of SAN 619 tech. on sediment-dwelling Chironomus riparius (syn. Chironomus thummi) under static conditions Novartis Crop Protection AG, Basel, Switzerland Novartis Crop Protection AG, Basel, Switzerland, Report No 983753 GLP Not Published Syngenta File N° SAN619/0627
Hamburger, F.	1985	SAN 619 F - Fish toxicity in the carp Novartis Crop Protection AG, Basel, Switzerland Sandoz AG, Basel, Switzerland, Report No 149/85 GLP Not Published Syngenta File N° SAN619/5961
Hawkins, D.R.	1988	Soil adsorption and desorption of 1,2,4-Triazole. Novartis Crop Protection AG, Basel, Switzerland Rohm and Haas, Philadelphia, United States, Report No 34S-88-27 GLP Not Published Syngenta File N° CGA71019/0014
Heimbach, F.	1986	Acute toxicity of 1,2,4-triazole (technical) to earthworms. Novartis Crop Protection AG, Basel, Switzerland Bayer AG, Leverkusen, Germany, Report No HBF/RG 59 GLP Not Published Syngenta File N° CGA71019/0021
Hertl J., Breitwieser H.	2003	AcuteToxicity of (1H-1,2,4-triazol-1-yl)acetic acid to Rainbow Trout (Oncorhynchus mykiss) in a 96-hour Static Test. Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, TM92 14361230 GLP, not published Syngenta File No CGA142856/0025
Hertl J., Breitwieser H.	2003a	AcuteToxicity of (1H-1,2,4-triazol-1-yl)acetic acid to Daphnia magna in a 48-hour Immobilization Test. Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, TM93 14362220 GLP, not published Syngenta File No CGA142856/0026
Hertl J., Breitwieser H.	2003b	Toxicity of (1H-1,2,4-triazol-1-yl)acetic acid to Scenedesmus subspicatus in an Algal growth Inhibition test. Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, TM97 14363210 GLP, not published Syngenta File No CGA142856/0021
Jenkins, C.A.	1989	SAN 619 F - 21-day rainbow trout toxicity study under flow-through exposure conditions Novartis Crop Protection AG, Basel, Switzerland Life Science Research Ltd., Eye, United Kingdom, Report No 89/SAS032/0197 GLP Not Published Syngenta File N° SAN619/5962

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Jenkins, C.A.	1993	SAN 619 F : Determination of EC50 to Chlorella vulgaris (72 hour static assay) Novartis Crop Protection AG, Basel, Switzerland Life Science Research Ltd., Eye, United Kingdom, Report No 93/SAS049/0830 GLP Not Published Syngenta File N° SAN619/5314
Klein S., Rosenkranz B.	2002	Toxicity of (1H-1,2,4-triazol-1-yl)acetic acid on Reproduction of the Collembola Folsomia candida in artificial Soil Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, TM91 Project 14366016 GLP, not published Syngenta File No CGA142856/0022
Leopold, M.A.	1993	Acute oral toxicity study with cyproconazole in Bobwhite quail Novartis Crop Protection AG, Basel, Switzerland NOTOX B.V., 'S Hertogenbosch, Netherlands, Report No 096558 GLP Not Published Syngenta File N° SAN619/0105
Luhrs U.	2002	AcuteToxicity (14 days) of (1H-1,2,4-triazol-1-yl)acetic acid to the Earthworm Eisenia fetida in Artifical Soil. Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, TM81 Project 14364021 GLP, not published Syngenta File No CGA142856/0024
Mead-Briggs, M.	1990	An evaluation of the residual effects of the fungicides Alto 100 SL and Alto Elite to the parasitic wasp, Aphidius rhopalosiphi Novartis Crop Protection AG, Basel, Switzerland Agrochemical Evaluation Unit, The University, Southampton, United Kingdom, Report No SAN-90-1 GLP Not Published Syngenta File N° SAN619/0108
Mead-Briggs, M.	1995	An Extended Laboratory Test to Evaluate the Side-Effects of the Fungicide ALTO 100 SL on Adults of the Parasitic Wasp Aphidius rhopalosiphi, when Applied to Barley Plants Novartis Crop Protection AG, Basel, Switzerland Report No SAN-95-1 GLP Not Published Syngenta File N° SAN619/6683
Meister, A, Klein, S	2002	Effects of SAN 619 formulated as SL 100 (A-9898 A) on the Decomposition of Organic Material enclosed in Litter Bags in the Field Syngenta Crop Protection AG, Basel, Switzerland IBACON GmbH, Rossdorf, Germany, Report No 10741081 GLP Not Published Syngenta File N° SAN619/7201

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8 ANNEXES