Annex XV dossier

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Epoxiconazole

EC number: 406-850-2

CAS number: 133855-98-8 (formerly: 106325-08-0)

Submitted by: Swedish Chemicals Agency

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CONTENTS

TABLES AND FIGURES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Epoxiconazole

EC Number: 406-850-2

CAS number: 133855-98-8 (formerly: 106325-08-0)

Note: Different CAS numbers are used for epoxiconazole. CAS number **135319-73-2**, which is used by e.g. EFSA for epoxiconazole, is the collective number for all stereoisomers, whereas CAS number 133855-98-8 is the number used for the isomer included in Annex I of Dir 67/548/EEC. Both CAS numbers are used, often together without further definition, for epoxiconazole by industry, e.g. in many Safety Data Sheets. According to the rapporteur country of the Draft Assessment Report (DAR) for epoxiconazole, Germany, the substance that is evaluated in the DAR has CAS number 133855-98-8 (formerly: 106325-08-0), and the list of end-points (Reference 1) concerns CAS number 133855-98-8, not 135319-73-2 as is stated in the document. However, it should be noted that the EC number (406-850-2), which correctly corresponds to CAS number 133855-98-8, is often used together with CAS number 135319-73-2. It should also be noted that the chemical names used for CAS number 135319-73-2 differs. According to STN the correct chemical names for each CAS number are:

133855-98-8: 1H-1,2,4-Triazole, 1-[[(2R,3S)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-2-oxiranyl]methyl]-

135319-73-2: 1H-1,2,4-Triazole, 1-[[3-(2-chlorophenyl)-2-(4-fluorophenyl)-2-oxiranyl]methyl]-

Registration number (s): -

Purity: minimum 920 g/kg

Impurities: There are a number of impurities claimed as confidential by the producer (see Technical dossier in IUCLID 5, section 1.4)

Proposed classification based on Directive 67/548/EEC criteria:

Repr.Cat. 2; R61 (May cause harm to the unborn child)

(Note: No change to the current classification with Repr. Cat. 3; R62, Carc. Cat. 3; R40 and N; R51-53 is proposed.)

Proposed classification based on GHS criteria:

Repr 1B; H360D (May damage the unborn child)

(Note: No change to the current classification with Repr 2; H361f; Carc 2; H351; and H411 is proposed.)

Proposed labelling:

T; R61

JUSTIFICATION

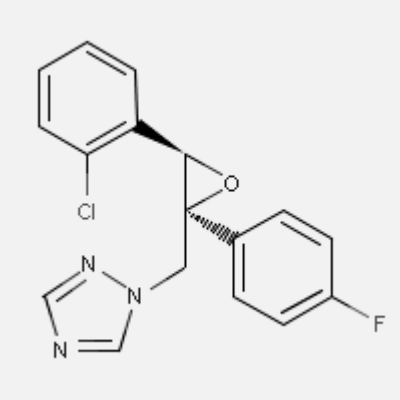
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical name:	1H-1,2,4-Triazole,1-[[(2R,3S)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-2-oxiranyl]methyl]-
EC name:	
CAS number:	133855-98-8 (formerly: 106325-08-0) (Note: see p. 4)
IUPAC name:	(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane

1.2 Composition of the substance

There are a number of impurities stated as confidential by the producer (see Technical dossier in IUCLID 5, section 1.2)

Chemical name:	1H-1,2,4-Triazole,1-[[(2R,3S)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-2-oxiranyl]methyl]-
EC number:	406-850-2
CAS number:	133855-98-8 (formerly: 106325-08-0) (Note: see p. 4)
IUPAC name:	(2RS,3RS)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-[(1H-1,2,4-triazol-1-yl)methyl]oxirane
Molecular formula:	$C_{17}H_{13}ClFN_3O$
Structural formula:	
Molecular weight:	329.76 g/mol
Typical concentration (% w/w):	minimum 920 g/kg minimum
Concentration range (% w/w):	

1.3 Physico-chemical properties

Table 1. Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Comment/reference
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	colourless solid (99.9% purity)	Reference 1.
VII, 7.2	Melting/freezing point	3.2	136.2-137°C (99.9% purity)/no data	Reference 1.
VII, 7.3	Boiling point	3.3	not applicable	Reference 1.
VII, 7.5	Vapour pressure	3.6	< 1.0 * 10-5 Pa (20°C; 99.1% purity)	Reference 1., extrapolated from measurements at 70°C
VII, 7.6	Surface tension	3.10	68.7 mN/m (0.5% w/w; 99.1% purity) 72.9 mN/m (6.4 mg/L; 99.6% purity)	Reference 1.
VII, 7.7	Water solubility	3.8	7.1 mg/L (deionized water)	Reference 1.
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	log PO/W=3.3	Reference 1.
VII, 7.10	Flammability	3.13	not considered highly flammable (93.7% purity)	Reference 1.
VII, 7.11	Explosive properties	3.14	no thermal or mechanical sensitivity with respect to shock or friction was observed (93.7% purity)	Reference 1.
VII, 7.12	Self-ignition temperature		shows no self-ignition up to 400°C (93.7% purity)	Reference 1.
VII, 7.13	Oxidizing properties	3.15	no oxidizing properties	Reference 1., theoretical assessment
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17		
XI, 7.16	Dissociation constant	3.21	does not dissociate in water, no pKa value could be determined (99.9% purity)	Reference 1.

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Epoxiconazole is used as a fungicide having protective, curative and eradicative effects. Intended use is under field conditions in agriculture, e.g. on cereals and sugar beets as protection against leaf spot diseases.

2.3 Uses advised against

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Carc. Cat. 3; R40

Repr. Cat. 3; R62-63

N; R51-53

Annex I Index no.: 613-175-00-9

3.2 Self classification(s) -

4 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Excretion of ^{14}C-BAS 480 F (epoxiconazole) after oral administration to male and female Fisher rats was tested at nominal dose levels of 3 and 100 mg/kg bw. The excretion was almost complete at 168 h post-dosing, with 90-95 % of the administered dose being excreted within 72 h. It occurred mostly via faeces (76 % - 82 %) and urine (12 % - 21 %), while in expired air, no significant amounts of radioactivity were detected. Quantitatively, there were no significant differences between sexes, between the low- (ca. 3 mg/kg bw) and high-dose (ca. 100 mg/kg bw) groups, or after repeated oral administration (14 x unlabelled and 1 x labelled epoxiconazole at the low dose). However, on the whole, excretion occurred later with female animals than with the males.

Although the results have to be used with care due to experimental drawbacks, it was found that biliary excretion played an important role in the elimination of epoxiconazole, but, quantitatively, a pronounced difference was observed between males, in which up to 70 % (low dose) of the administered radioactivity were secreted with the bile, and females with a maximum of 34 % biliary excretion at the low dose within the first 21 h.

Based on urinary and biliary elimination, oral absorption was about 80 % in male and 50 % in female animals.

Pharmacokinetics

Peak plasma concentrations were observed no later than 2 h after administration in both dose groups and with both sexes. Both C_{max} and AUC's of the females were about 50 % higher at the low, and 10 - 20 % higher at the high-dose, compared to those of the males. For both sexes, C_{max} increased slightly less than proportionally with dose, whereas the AUC was linear over the tested dose range, indicating that the absorption process was not saturated at the high dose level. Terminal half-lives of 5 - 6 and 32 - 34 h were observed for the low and high dose, respectively.

Distribution

Epoxiconazole was widely distributed in the organism, with highest residues found in blood, liver, kidneys, spleen, lung, and adrenals. Overall, at 168 h post-dosing, only small amounts of radioactivity were detected in these organs, while whole blood (but not plasma) and spleen levels were declining more slowly, indicating some kind of binding to blood cells by either the parent substance or its metabolites (some effort was undertaken to prove the latter, but interpretation of results was seen as questionable).

Taking together the results of both the tissue distribution and pharmacokinetics experiments, it was concluded that epoxiconazole is unlikely to accumulate in tissues. On the other hand, very slow elimination from blood was observed, with terminal half-lives of more than 100 h at the high dose.

Metabolism

After oral administration to male and female rats, epoxiconazole was rapidly and intensively metabolised to a large number of biotransformation products. Phase I biotransformation is

characterised by the hydrolytic opening of the oxirane ring, hydroxylation of the chlorophenyl ring and - to a lesser extent - also of the fluorinated aromatic ring. In addition, cleavage of the carbon bridge between the two aromatic nuclei is observed.

Quantitatively the most important phase II reactions consist of the formation of glutathion adducts. This includes addition at the chlorophenyl ring, the substitution of aromatic chlorine as well as the opening of the oxirane ring and formation of arene oxides. Degradation of these glutathion adducts further enlarges the number of metabolites.

No major differences were observed with regard to sex and dose level.

Dermal absorption

The dermal absorption of epoxiconazole *in vivo* was determined by applying test solutions of 1 and 10 g/L (about 10-fold less concentrated than the final preparation of 125 g/L), respectively, to the skin of male and female Fisher rats, resulting in applied concentrations of 0.06 and 0.6 mg/cm^2 (equivalent to doses of about 3.7 and 37 mg/kg bw). After an exposure duration of 10 h, absorbed amounts were ca. 8 % of dose for the lower and ca. 16 % of dose for the higher concentration.

Dermal penetration through rat skin *in vitro* was found to be about 2 - 3 times higher than observed in human skin at 0.0125 and 0.1 mg/cm^2, and about 5 - 7.5 times higher at 0.8 mg/cm^2, respectively.

Overall, a dermal absorption of 3 % was established.

(Draft Assessment Report, Reference 2)

5.2 Acute toxicity

5.2.1 Summary and discussion of acute toxicity

Table 2. Summary of studies on acute toxicity

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Acceptability	Conclusions	Comments
Hildebrand B., Kirsch P. 1988(a), TOX2003-1814, Reg. No. 1988/0107	Acute oral, Wistar rat, acceptable	LD_{50}(m) = 5000 mg/kg bw LD_{50}(f) > 5000 mg/kg bw LD_{50}(m+f) = 5000 mg/kg bw	Unspecific symptoms, mortality at ≥ 3160 mg/kg bw after 3 days or later
Hildebrand B., Kirsch P. 1988(b), TOX2003-1815, Reg. No. 1988/0108	Acute dermal, Wistar rat, acceptable	LD_{50}(m+f) > 2000 mg/kg bw	No systemic toxicity, no local irritation
Klimisch H.-J. *et al*. 1988, TOX2003-1819, Reg. No. 1988/0081	Acute inhalation, Wistar rat, acceptable	LC_{50}(m+f) > 5.3 mg/L/4 h (dust aerosol)	Signs of airway irritation during and shortly after exposure; no symptoms thereafter, no mortality; MMAD = 3.9 µm

In summary, epoxiconazole proved to be of low acute toxicity. In the test for acute oral toxicity in rats, mortality rates were smaller than 50 % at all of the examined dose levels and thus no meaningful statistical LD_{50} calculation was possible. However, while only 1 female of the high-dose group died in the test, 2/5 males died in both the 3160 and 5000 mg/kg bw dose groups. Therefore 5000 mg/kg bw was set as the overall acute oral LD_{50}. Toxic symptoms were unspecific and mortality occurred late (day 3 or later within the 21-day observation period).

No toxic effects of epoxiconazole were observed after dermal application to Wistar rats, with an LD_{50} value above the limit dose of 2000 mg/kg bw, which caused neither mortality nor systemic toxicity. In addition, no local reaction was observed at the application site.

The inhalation toxicity of epoxiconazole in Wistar rats proved to be low (LC_{50} > 5.3 mg/L/4 h). There was only a slight irritation of the airways during exposure and shortly thereafter in the dust aerosol study. No mortality was observed in this study. No classification for acute toxicity is proposed.

For further details, see Draft Assessment Report, Reference 2.

(Draft Assessment Report, Reference 2)

5.3 Irritation

Not evaluated for this dossier.

5.4 Corrosivity

Not evaluated for this dossier.

5.5 Sensitisation

Not evaluated for this dossier.

5.6 Repeated dose toxicity

5.6.1 Summary and discussion of repeated dose toxicity

Table 3. Summary of repeated dose studies

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels, Acceptability	Comments
4-week oral toxicity		
Schilling K. *et al*. 1989b, TOX2003-1821, Reg. No. 1991/10889	4-week dietary (range-finding), Wistar rats Chbb = THOM (SPF), 0; 250; 1000; 4000 ppm, acceptable	<u>4000 ppm</u> Reduced food consumption, severely reduced body weights. Anaemic effects with compensatory reactions. Decreased adrenal weights with lipid deposits in males and a regressive transformation of the outer cortex in females <u>1000 ppm and above</u> Clinical chemistry indicating liver toxicity. Increased liver weights, with hepatocellular hypertrophy. <u>250 ppm and above</u> Increased liver γ-GT activity
Hellwig J. *et al*. 1990a, TOX2003-1828, Reg. No. 1989/0496	4-week dietary (range finding), beagle dogs, 0; 400; 1600; 3200; 6400 ppm, acceptable for range-finding	<u>6400 ppm</u> Mortality, clinical signs, body weight loss <u>3200 ppm and above</u> Reduced feed consumption, clinical signs (vomiting) <u>1600 ppm and above</u> Altered clinico-chemical parameters in males <u>400 ppm and above</u> Increased relative liver weight, reduced body weight gain in females, raised AP and γ-GT

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels, Acceptability	Comments
13-week oral toxicity		
Schilling K. *et al*. 1991a, TOX2003-1823, Reg. No. 1991/10836	90-day dietary, Wistar rats Chbb=Thom(SPF), 0; 30; 90; 270; 800 ppm, acceptable	<u>800 ppm</u> Altered clinico-chemical parameters. Increased relative liver weights in males and females, increased absolute liver weight in females. <u>270 ppm and above</u> Hepatocellular hypertrophy in both sexes, increased serum and liver homogenate γ-GT
Schilling K. *et al*. 1991b, TOX2003-1824, Reg. No. 1991/10837	90-day dietary (range-finding), Wistar rats Chbb=Thom(SPF), 0; 500; 1000; 1500; 2000 ppm, supplementary	<u>2000 ppm</u> Reduced feed consumption – males, decreased relative adrenal weight and regressive transformation of the adrenal cortex– females <u>1500 ppm and above</u> Reduced body weight gain – males. Lipoid deposits in adrenal cortex – females <u>1000 ppm and above</u> Altered clinico-chemical parameters. Increased liver weight – both sexes <u>500 ppm and above</u> Increased liver weights – females, decreased adrenal weights – males
Schilling K. *et al*. 1991c, TOX2003-1825, Reg. No. 1991/10908	90-day dietary, C57BL/6NCrlBR mice, 0; 7.5; 125, 250, 500; 1000 ppm, acceptable	<u>1000 ppm</u> Liver cell degeneration – females <u>500 ppm and above</u> Reduced body weight gain – males; liver cell degeneration – males <u>125 ppm and above</u> Altered haematological and clinico-chemical parameters; increased liver weights with centrilobular hepato-cellular hypertrophy
Schilling K. *et al*. 1991d, TOX2003-1826, Reg No. 1991/10855	90-day dietary, B6C3F1 mice, 0; 30; 90; 270 ppm, acceptable	<u>270 ppm</u> Altered clinico-chemical parameters in males and females; impaired liver function in males and females; liver cell degeneration in some males; centrilobular hepatocellular hypertrophy in males and females. <u>90 ppm</u> Impaired liver function in males; centrilobular hepatocellular hypertrophy in males. <u>30 ppm and above</u> Increased relative liver weights

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels, Acceptability	Comments
Schilling K. *et al*. 1991e, TOX2003-1827, Reg. No. 1991/10856	90-day dietary, B6C3F1 mice, 0; 7.5; 15; 30 ppm, supplementary	<u>30 ppm</u> Reduced triglycerides and cholesterol and increased absolute and relative liver weights.
Hellwig J. *et al*. 1990b, TOX2003-1829, Reg. No. 1990/0411	90-day dietary, beagle dogs, 0; 50; 200; 800 ppm, acceptable	<u>800 ppm</u> Minimal to moderate hypertrophy of renal proximal tubular epithelial cells in males and in one single female <u>200 ppm and above</u> Minimal hypertrophy of renal proximal tubular epithelial cells – males
12-month oral toxicity		
Mellert W., Hildebrand B. 1992c, TOX2003-1830, Reg. No. 1992/10687	12-month dietary, beagle dogs, 0; 50; 500; 1500 ppm, acceptable	<u>1500 ppm</u> Mortalities, considered to be related to liver damage <u>500 ppm and above</u> Altered clinico-chemical parameters in both sexes; hepatitis <u>50 ppm and above</u> Reduced red blood cell parameters in males
Mellert W., Hildebrand B. 1992d, TOX2003-1831, Reg. No. 1992/10690	12-month dietary, male beagle dogs, 0; 10; 20; 30; 40 ppm, supplementary	No test substance related changes
21-d dermal toxicity		
Kirsch P. *et al*. 1992, TOX2003-1832, 1992/10691	21-day dermal, Wistar rats Chbb=Thom(SPF), 0; 100; 400; 1000 mg/kg bw/d, acceptable	<u>1000 mg/kg bw/d</u> Reduced red blood cells and haematocrit; increased absolute and relative liver weights in both sexes with slight centrilobular hepatocellular hypertrophy in males. No signs of local irritation

In conclusion, the repeated dose oral toxicity of epoxiconazole is characterized by effects on the liver in all three species tested. At high dose levels, liver function was impaired resulting in signs of liver toxicity. At lower dose levels, only liver weight increases were seen. In rats and mice, also the adrenal gland was a target organ. Histopathology demonstrated lipoid deposits as well as regressive transformation in female rats. In dogs, minimal to moderate hypertrophy of the proximal tubular epithelial cells of the kidneys was observed. At very high dose levels in the 4-week studies in rats and mice, signs of anaemia were noted.

No classification is proposed.

For further details, see Draft Assessment Report (Reference 2).

(Draft Assessment Report, Reference 2)

5.7 Mutagenicity

5.7.1 In vitro data

Table 4. Summary of *in vitro* studies on mutagenicity

Author(s)/Year, RMS Report ID, Company Report ID	Test system/ Acceptability	Strain/species	Test conditions	Result
In vitro				
Engelhardt G., Hoffmann H. D. 1987, TOX2003-1833, Reg. No. 1987/0423	Point mutation in bacterial cells/ Ames test, acceptable	S. typhimurium TA 1535, TA 100, TA 1537, TA 98, Standard plate and preincubation technique	With S9 mix Without S9 mix 20 – 5000 µg/plate	Negative Negative
Engelhardt G., Hoffmann H. D. 1989, TOX2003-1834, Reg. No. 1989/0297	Point mutation in bacterial cells/ Ames test, acceptable	E. coli WP2 uvrA, Standard plate and preincubation technique	With S9 mix Without S9 mix 20 – 5000 µg/plate	Negative Negative
Young R. R. 1990, TOX2003-1837, Reg. No. 1990/0271; Amendments: TOX2003-1838, Reg. No. 1993/11081; TOX2003-1839, Reg. No. 1994/10731	Point mutation in mammalian cells, acceptable	Chinese hamster ovary (CHO) cells (HPRT locus)	With S9 mix Without S9 mix 50 – 1000 µg/mL	Negative Negative
Heidemann A. 1989, TOX2003-1840, Reg. No. 1989/0369	Chromosome aberration in mammalian cells, acceptable	Chinese hamster ovary (CHO) cells	With S9 mix Without S9 mix 10; 50; 140 µg/mL	Negative Negative
Fautz R. 1991, TOX2003-1842, Reg. No. 1991/10833	Unscheduled DNA synthesis, acceptable	Rat primary hepatocytes	0.15 – 150 µg/mL	Negative

Negative results were obtained in the Ames test with four *S. typhimurium* strains and one *E. coli* strain.

Point mutations in mammalian cells were assessed in Chinese hamster ovary cells (HPRT assay). In this study, no mutagenic effects were observed either with or without metabolic activation.

In the chromosome aberration test using Chinese hamster ovary cells, no increase in mutation frequency was noted in the presence or absence of metabolic activation.

DNA damage and repair, investigated *in vitro* using the UDS test in primary rat hepatocytes, showed no influence of epoxiconazole.

(Draft Assessment Report, Reference 2)

5.7.2 In vivo data

Table 5. Summary of *in vivo* studies on mutagenicity

Author(s)/Year, RMS Report ID, Company Report ID	Test system/ Acceptability	Strain/species	Test conditions	Result
In vivo				
Engelhardt G., Hoffmann H. D. 1991, TOX2003-1835, Reg. No. 1991/10314	Micronucleus test, acceptable	NMRI mice	Oral (gavage - single application): 0; 200; 1000; 5000 mg/kg bw/d	Negative
Lutz W. K. *et al*. 1992, TOX2003-1843 Reg. No. 1992/10923	DNA-adduct formation, not acceptable	Wistar rats and C57Bl mice	1,500/500 ppm for 24 d via the diet, followed by ^{14}C-epoxiconazole via gavage: 131/27.8 mg/kg bw/d (rat/mouse)	Not assessable

In vivo, no indication of a clastogenic or spindle poisoning effect was observed in the mouse micronucleus test after a single application of epoxiconazole by gavage.

(Draft Assessment Report, Reference 2)

5.7.3 Human data

5.7.4 Other relevant information

5.7.5 Summary and discussion of mutagenicity

Epoxiconazole was evaluated for possible mutagenic/genotoxic effects *in vitro* and *in vivo* in five different tests covering all endpoints of genetic damage. None of the genotoxicity studies contains data that confirm the stability of epoxiconazole in the solvent DMSO or in the incubation media. The notifier stated that a representative batch has been analysed, but not where the results of this test were to be found.

The studies cover all endpoints required for mutagenicity and genotoxicity testing. It is concluded that epoxiconazole has no mutagenic or genotoxic potential, and hence no classification is proposed.

For further details, see Draft Assessment Report, Reference 2.

(Draft Assessment Report, Reference 2)

5.8 Carcinogenicity

5.8.1 Summary and discussion of carcinogenicity

Table 6. Summary of studies on chronic toxicity and carcinogenicity

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels, Acceptability	Comments
Mellert W., Hildebrand B. 1992e, TOX2003-1844 Reg. No. 92/10685	24-month feeding, Chbb:THOM (SPF) Wistar rats, 0, 30, 150, 750, 1500 ppm, acceptable	<u>1500 ppm</u> Males and females: reduced food consumption and body weight gain, increased liver weights <u>750 ppm and higher</u> Males: transiently reduced body weight gain and food consumption Females: ovarian cysts Males and females: reduced red blood cell parameters, altered clinical chemistry parameters, hepatocellular hypertrophy <u>150 ppm and higher</u> Males: reduced platelets Females: reduced triglycerides Not oncogenic
Mellert W., Hildebrand B. 1992f, TOX2003-1845, Reg. No. 92/10686	24-month feeding carcinogenicity, Chbb:THOM (SPF) Wistar rats, 0, 30, 150, 750, 1500 ppm, acceptable	<u>1500 ppm</u> Males and females: increased liver weights Females: hepatocellular hypertrophy, increased incidence of adrenal gland cortex tumours Males: increased incidence and severity of fatty change in the liver, reduced incidence of Leydig cell tumours <u>750 ppm and higher</u> Males and females: reduced body weight gain, reduced food consumption Females: increased relative liver weight, increased incidence of ovarian theca granulosa cell tumours Males: hepatocellular hypertrophy, eosinophilic and mixed cell foci in liver <u>150 ppm and higher</u> Females: decreased incidence and severity of fatty change in the liver, increase of ovarian cysts
Mellert W., Hildebrand B. 1992, TOX2003-1846, Reg. No.92/10699	18-month feeding carcinogenicity, C57BL mice, 1, 5, 200, 500 (males only) and 1000 ppm (females only),	<u>1000 ppm</u> Females only: focal liver necrosis and liver hyperplasia, eosinophilic foci in the liver, increased deposition of amyloid in several organs (liver, ovaries), increased incidence of liver tumours <u>500 ppm</u> Males only: focal liver necrosis and liver hyperplasia,

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels, Acceptability	Comments
	acceptable	increased incidence of liver tumours 200 ppm and higher Males and females: reduced body weight gain, increased liver weights Males: eosinophilic foci in liver, increased deposition of amyloid in testes

The chronic toxicity/oncogenicity studies with epoxiconazole include a 24-month chronic feeding study and a 24-month oncogenicity study with Wistar rat as well as an 18-month feeding study with C57BL mice.

Chronic toxicity study

Rat

In the chronic toxicity study in rats, the test substance reduced body weight development in males and females of the 1500 ppm group. A transient reduction of body weight development was seen in 750 ppm males. Food consumption was also affected in these groups. A reduction in some red blood cell parameters was observed at the high dose level in males and females as well as in females at 750 ppm. Platelets were reduced at 150 ppm and above in males and at 750 ppm and above in females. Clinical chemistry findings indicating the liver as a target organ were seen predominantly at 1500 ppm, the most sensitive parameter being a reduction of triglycerides at 150 ppm and above in females. Liver weights were noticeable increased only at the high dose level whereas an increase in the incidence and severity of hepatocellular hypertrophy was seen already at 750 ppm. Females at 750 and 1500 ppm exhibited increased cyst formation of the ovary. Epoxiconazole was not oncogenic in this study.

Carcinogenicity studies

Rat

In the carcinogenicity study in rats, slight decrements in food consumption and body weight gain resulted in slight to moderately lower body weights for males and females at 750 ppm and 1500 ppm after 24 months of dietary administration of epoxiconazole. The liver was identified as a target organ exhibiting non-neoplastic changes such as weight increases, hepatocellular hypertrophy and increased incidence of eosinophilic and mixed cell foci (males) at doses of 750 ppm and 1500 ppm. Decreased fatty change was seen in the females dosed with 150 ppm and higher, while increased fatty change was noted in males at 1500 ppm.

Increased incidences of adrenal gland cortex neoplasms were observed in 1500 ppm males and females. A dose-related increase in the number of females with ovarian cysts was found at 150 ppm and above while increased incidences of ovarian theca granulosa cell tumours were seen at 750 ppm and 1500 ppm.

Decreased incidences of neoplasms were noted for the testes (Leydig cell tumours) in 1500 ppm males, the adrenal gland medulla (phaeochromocytomas) in 1500, 750 and 150 ppm males and the pituitary gland (adenomas) in the 1500 ppm females.

The findings are considered indicative of an effect on the synthesis or availability of steroid hormones.

Mouse

In the carcinogenicity study in mice, treatment with epoxiconazole resulted in a decrease in body weight gain of males and females at a food concentration of 200 ppm and above. For the high dose the reduction in body weight amounted to 15 - 20 % at the end of the study. The target organ was the liver as indicated by an increased organ weights in high dose and intermediate dose females (1000 ppm and 200 ppm) and males (500 ppm and 200 ppm). Moreover, histopathological changes such as hypertrophy, hyperplasia and focal necrosis were present in high dose males and females. An increased incidence of eosinophilic liver cell foci was seen in high dose males and females as well as in 200 ppm males. There was a test substance-related increase in the incidence of liver neoplasia in the 1000 ppm females and 500 ppm males. In all other treatment groups there was no statistically or biologically relevant increase in liver neoplasia and no increase of any other neoplasia was observed in any treatment group.

Overall, it can be concluded that, in both species tested, one of the target organs for epoxiconazole-induced toxicity is the liver as indicated by increases in organ weights and histopathological changes. Clinical chemistry changes that are likely to be associated with alterations of liver function and adverse effects on red blood cell parameters were observed in rats. In addition, the studies identified male and female gonads as (possibly secondary) targets of epoxiconazole toxicity. Inhibition of enzyme(s) involved in the synthesis of steroid hormones and an induction of liver enzymes are considered the possible mechanisms that affect the endocrine system.

Concerning the neoplastic potential of the test substance, increased tumour incidences (female rats - 1500 ppm: adrenal gland cortex and ovarian theca granulosa cells; male mice – 500 ppm: liver cell tumours; female mice – 1000 ppm: liver cell tumours) were observed only at dose levels that also resulted in significantly lower body weights at the end of the exposure period. Since the genotoxicity studies did not identify a mutagenic potential of epoxiconazole epigenetic mechanisms are considered to be responsible for tumour formation.

For further details, see Draft Assessment Report, Reference 2.

(Draft Assessment Report, Reference 2)

Note: Epoxiconazole is currently classified as a carcinogen category 3 (Carc. Cat. 3; R40) in Annex I of Directive 67/548/EEC and no change this classification is proposed.

5.9 Toxicity for reproduction

Studies included in the Draft Assessment Report (DAR) and hence in the first classification decision process:

Fertility/Developmental toxicity:

- Hellwig & Hildebrand, 1989 (oral, rat), p. 20ff
- Hellwig & Hildebrand, 1990b (oral, rat), p. 24ff
- Hellwig & Hildebrand, 1992 (oral, rat), p. 27ff
- Schneider *et al*, 2002 (oral, rat), p. 33ff
- Hellwig & Hildebrand, 1990a (oral, rabbit), p. 43ff
- Hellwig & Hildebrand, 1993 (dermal, rat), p. 46ff

Endocrine disruption:

- Mellert, 1992 (*in vivo*, rat), p. 48ff
- Wuttke, 1995 (*in vitro*), p. 54ff
- Wuttke, 2001 (*in vitro*), p. 57ff

Maternal toxicity:

- Schneider *et al*, 2001, p. 63ff

New studies not included in the DAR:

Fertility/Developmental toxicity:

- Taxvig *et al*, 2007 (oral, rat), p. 40ff

Endocrine disruption:

- Taxvig *et al*, 2007 (*in vivo*, rat), p. 62 (40ff)
- Birkhøj Kjaerstad *et al*, 2007 (*in vitro*), p. 60ff

Summary table of all studies on p. 67ff

5.9.1 Effects on fertility

See 5.9.2 Developmental toxicity

Note: Epoxiconazole is currently classified as a Repr. Cat. 3; R62 in Annex I of Directive 67/548/EEC and no change this classification is proposed.

5.9.2 Developmental toxicity

5.9.2.1 Oral exposure

Rat

Report:	Hellwig J., Hildebrand B. 1989;TOX2003-1848 Study of the prenatal toxicity of Reg. No. 205 259 in rats after oral administration (gavage) range finding study BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF DocID 89/0477
Data taken from:	Draft Assessment Report, Reference 2.
GLP:	Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)
Guideline:	EPA 83-3, OECD 414, JMAFF
Deviations:	Foetuses not examined for visceral and skeletal changes (only gross examination performed).
Acceptability:	The study is considered to be acceptable as a range-finding experiment.

Material and Methods

Test material: Epoxiconazole, purity 92.8 %, batch N 33

Test animals: groups of 25 female Wistar Chbb:THOM (SPF) rats, provided by Karl Thomae, Biberach, Germany

In order to establish dose levels for the main study, epoxiconazole was examined for its prenatal toxicity in Wistar rats. The dams were treated from day 6 through day 15 post coitum (p.c.) with epoxiconazole doses of 0 (control treated with the vehicle double distilled water with 0.5 % carboxymethyl cellulose); 20; 60 and 180 mg/kg bw/d by gavage at a constant dosing volume of 5 mL/kg bw.

Food consumption and body weights were recorded regularly throughout the study period. The animals' state of health was checked daily. On day 20 p.c., all females were sacrificed and assessed by gross pathology. The foetuses were dissected from the uterus, sexed, weighed and examined externally.

Findings

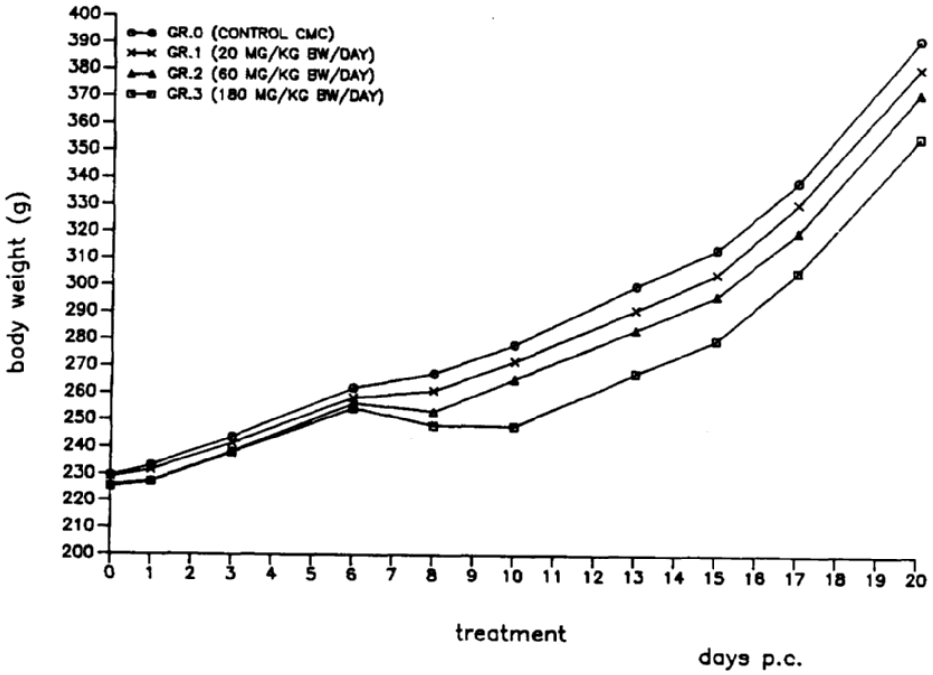
Food consumption and body weight gain were reduced during the treatment period (see Table 7 and Figure 1). At the mid and high dose levels an initial body weight loss was observed in addition to reduced weight gains during and following the treatment period. A reduced corrected body weight gain from day 6 until termination was also seen at the lowest dose level. Clinical symptoms were only observed at the high dose level where several dams had a reddish nasal discharge, piloerection and/or fur smeared with urine.

Table 7. Maternal data - prenatal toxicity range finding gavage study in Wistar rats

Parameter	Dose level (mg/kg bw/d)			
	0	20	60	180
Mated females on study	25	25	25	25
Pregnant females on study	24	23	20	23
Clinical symptoms:				
Piloerection	0	0	1	8
Reddish nasal discharge	0	0	0	2
Urine-stained fur	0	0	0	2
Mortality	0	0	0	0
Food consumption treatment day 6-15 (g/animal/day and % of control)	26.6 100 %	24.9 94 %	23.2 87 %	19.5* 73 %
Body weight (see also Fig, 1)			Reduced days 8-17*/**	Reduced Days 8-20 **
Body weight gain days 6-15 (g and % of control)	51.8 100 %	46.7 90 %	40.3** 78 %	25.8** 50 %
Corrected (net) body weight gain from day 6 until termination (g and % of control)	52.7 100 %	44.2* 84 %	40.3** 76 %	27.7** 51 %

* $p < 0.05$ **$p < 0.01$

Figure 1. Body weight of pregnant dams (gram) in control and epoxiconazole treated groups (20, 80 and 180 mg/kg bw (as in Hellwig & Hildebrand, 1989)



A dose-dependent increase in post-implantation loss was seen, although this was not statistically significant (see Table 8). Placental weights were significantly increased at all dose levels and in a few cases coagulated blood was observed around the placenta (n.s.).

In high dose foetuses – which were only examined macroscopically in this range finding study - cleft palates were found in 136 out of 271 foetuses (50.2%) from 18 out of 20 litters (90%) examined. No foetal findings were observed at the mid dose level while in the low dose group 2 foetuses from 2 different litters exhibited multiple defects (1 posterior truncation, 1 craniofacial defects including cleft palate; n.s.). An increase in amniotic fluid was noticed in 10 foetuses of high dose litter.

Table 8. Data at caesarean section/foetal examination prenatal toxicity range finding gavage study in Wistar rats

Parameter	Dose (mg/kg bw/d)			
	0	20	60	180
Pregnant dams	24	23	20	23
Female mortality	0	0	0	0
Litter death	0	0	0	3
Premature birth	0	0	0	0
Corpora lutea; CL (mean)	15.5	14.9	14.9	15.5
Implantation sites (mean)	14.7	13.9	13.9	14.7
Preimpl. loss (> 2 CL)	2	3	2	1
Postimpl. loss (> 2 implants)	2	2	4	7
Total resorptions (mean)	1.3	0.9	1.9	2.9
Early resorptions (mean)	1.0	0.6	1.0	2.3
Late resorptions (mean)	0.3	0.3	0.9	0.6
Dead foetuses	0	0	0	0
Live foetuses (mean)	13.4	13.0	12.1	13.6
Placental weights in g (mean)	0.43	0.49**	0.58**	0.61**
Placental blood coagulum (litters/foetuses)	0	1/2	3/4	2/6
Foetal weights in g (mean)	3.9	4.1	4.0	4.0
Total malformations (litter incidence, %)	0 (0)	2 (8.7)	0 (0)	18 (90)**
Total malformations (foetal incidence, %)	0	2 (0.7)	0 (0)	136 (50.2)**

* $p<0.05$, **$p<0.01$

Conclusion

Epoxiconazole caused signs of maternal toxicity at the high dose level with respect to clinical symptoms, food consumption and body weight development. Food consumption and corrected body weight gain were affected down to the lowest dose level of the study (20 mg/kg bw/d), but was more pronounced at the highest dose. A slight increase in the number of dams with excessive post-implantation loss was seen although this was not significant. Placental weights were increased in all dose groups in a dose-dependent manner. Macroscopic evaluation of the foetuses revealed a very high incidence of foetuses with cleft palates, affecting 90 % of the litters in the high dose group. Based on the maternal toxicity findings it was concluded that the doses in the main study should not exceed 45 mg/kg bw/d.

(Draft Assessment Report, Reference 2)

Report: Hellwig J., Hildebrand B. 1990(b);TOX2003-1849
Study of the prenatal toxicity of Reg. No. 205 259 in rats after oral administration (gavage)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 90/0214

Data taken from: Draft Assessment Report, Reference 2.

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Guideline: EPA 83-3, OECD 414, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole, purity 93.2 %, batch CP 2431

Test animals: groups of 25 female Wistar Chbb:THOM (SPF) rats, provided by Karl Thomae, Biberach, Germany

Epoxiconazole was examined for its prenatal toxicity in Wistar rats. The dams were treated from day 6 through day 15 post coitum (p.c.) with epoxiconazole doses of 0 (control treated with the vehicle double distilled water with 0.5 % carboxymethyl cellulose), 5; 15 and 45 mg/kg bw/d by gavage at a constant dosing volume of 5 mL/kg bw.

Food consumption and body weights were recorded regularly throughout the study period. The animals' state of health was checked daily. On day 20 p.c., all females were sacrificed and assessed by gross pathology. The foetuses were dissected from the uterus, sexed, weighed and further investigated for external, soft tissue and/or skeletal changes.

Findings

Maternal data are compiled in Table 9 and caesarean section/foetal results in Table 10.

At the high dose level of 45 mg/kg bw/d, food consumption of dams was slightly reduced during treatment (especially on days 6 - 8 and 13 - 15). Body weight gain was impaired during the first days of treatment, but this did not result in any significant effects on corrected (net) bodyweight. No such findings were noted at the low and mid dose level.

Table 9. Maternal data - prenatal gavage toxicity study in Wistar rats

Parameter		Dose level (mg/kg bw/d)			
		0	5	15	45
Mated females on study		25	25	25	25
Pregnant females		21	24	23	22
Clinical symptoms		0	0	0	0
Mortality		0	0	0	0
Food consumption (g)[$]	days 6-8	23.8	23.6	23.6	20.8**
	days 13-15	28.1	27.5	27.3	26.1*
Body weight day 20 p.c. (g)		389.5	399.6	401.5	393.1
Body weight gain (g) during days 6-8[$]		6.8	5.7	5.4	1.2**
Corrected (net) body weight gain from day 6 until termination (g)		44.1	43.8	44.5	43.6

* $p<0.05$; ** $p<0.01$ [$] (no effect during other intervals)

The number of late resorptions was significantly increased (see Table 10) and consequently the late post-implantation loss was marginally increased at the high dose level. No increased embryolethality was noted at the low and mid dose level. Placental weights were significantly increased at the mid and high dose level. In the absence of detailed placental examinations, the toxicological significance of this finding is unclear; it may be related to an induction of placental metabolism either in the production of steroids or as an adaptive reaction to increased levels of epoxiconazole.

Foetal examination revealed a marked increase in the number of foetuses with skeletal variations, especially rudimentary cervical and/or accessory 14th rib(s) at the high dose level. The concomitant reduction of foetuses with short 13th ribs confirms a slight developmental shift in the determinations of vertebral elements at the thoracolumbar border. No other effects were noted with respect to foetuses at this dose level. No statistically significant foetal effects were observed at the low or mid dose level.

Table 10. Data at caesarean section/foetal examination prenatal toxicity gavage study in Wistar rats

Parameter		Dose level (mg/kg bw/d)			
		0	5	15	45
Females mated		25	25	25	25
Pregnant dams		21	24	23	22
Litter death		0	0	0	0
Premature birth		0	0	0	0
Corpora lutea, CL (mean)		16.5	17.5	16.9	16.8
Implantation sites (mean)		14.3	16.3	15.5	15.4
Preimpl. loss (> 2 CL)		6	4	3	2
Postimpl. loss (> 2 implants)		4	4	3	4
Late postimpl. loss (> 0)		3	3	5	10
Total resorptions (mean)		1.2	0.9	1.1	1.9
Early resorptions (mean)		1.0	0.8	0.8	1.3
Late resorptions (mean)		0.1	0.2	0.3	0.6*
Dead foetuses		0	0	0	0
Live foetuses (mean)		13.1	15.4	14.4	13.5
Placental weights in g (mean)		0.46	0.46	0.50*	0.59**
Foetal weights in g (mean)		3.9	3.8	3.9	3.9
Total malformations: litter incidence n (%)		2 (9.5)	4 (16.7)	4 (17.4)	5 (22.7)
Total malformations: foetal incidence n (%)		2 (0.7)	4 (1.1)	5 (1.5)	6 (2.0)
Total variations:	Foetal incidence n (%)	102 (37.1)	122 (33)	145 (43.7)	157 (52.9)**
	Affected foetuses/litter (%)	36.7	32.3	45.2	56.3**
Cervical ribs (litters/foetuses)		6/9	2/2	8/13	13/25*
Short 13th ribs (litters/foetuses)		6/15	8/20	6/15	3/6
14th rib rudiments (litters/foetuses)		0	1/2	6/7	15/39
Total retardations: Foetal incidence n (%)		64 (23.3)	83 (22.4)	79 (23.8)	77 (25.9)

* $p < 0.05$, ** $p < 0.01$

Conclusion

Epoxiconazole caused reduced food consumption and impaired body weight gain in dams at 45 mg/kg bw. Placental weights were increased at 15 and 45 mg/kg bw/d, however, this is not considered to be adverse at the 15 mg/kg bw/d dose level since foetal parameters remained in the normal range. Embryo-/foetotoxicity was observed at 45 mg/kg bw/d resulting in a slightly increased number of resorptions, marginally increased post-implantation loss and a markedly increased number of foetuses with skeletal variations (mainly supernumerary ribs).

(Draft Assessment Report, Reference 2)

Report: Hellwig J., Hildebrand B. 1992; TOX2003-1847
Reproduction study with Reg. No. 205 259 in rats. Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 92/10689

Data taken from: Draft Assessment Report, Reference 2.

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Guideline: EEC 87/302, EEC 67/548, EPA 83-4, OECD 416, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole, purity 93.2 %, batch CP 2431

Test animals: groups of 25 male and 25 female Wistar Chbb:THOM (SPF) rats, provided by Karl Thomae, Biberach, Germany

Epoxiconazole was administered to groups of 25 male and 25 female Wistar rats (F0 parental generation) in the feed at concentrations of 0; 10; 25 and 250 ppm. At least 70 days after the beginning of the treatment, F0 animals were mated to produce a first litter (F1a) and subsequently remated to produce a second litter (F1b retained only until weaning). Groups of 25 males and 25 females selected from F1a pups as F1 parental generation were offered diets containing 0; 10; 25 and 250 ppm of the test substance post-weaning, and the breeding program was repeated to produce F2 litter. The study was completed with the terminal sacrifice of F2 weanlings and F1 adult animals. Test diets containing epoxiconazole were offered continuously throughout the study.

Food consumption of the F0 and F1 parents was determined regularly during pre-mating (once weekly) and additionally during pregnancy and lactation periods. In general, body weights of the F0 and F1 parents were determined once weekly. However, females were weighed on days 0, 7, 14 and 20 of pregnancy, on the day of parturition, and on days 4, 7, 14 and 21 of the lactation period. Pups were weighed on the day after birth and on days 4, 7, 14 and 21 thereafter. Litter size was reduced to a maximum of 8 pups on postnatal day 4. The parents' and the pups' state of health was checked each day, and parental animals were examined for their mating and reproductive performances. Pups were sexed and monitored with respect to their development stages and their behaviour in certain tests. Their viability was recorded. All pups were examined macroscopically at necropsy; if necessary, certain pups were additionally inspected for organ/skeletal findings.

Blood samples were taken from 12 F0 and 12 F1 males and females of each test group towards the end of the treatment period for clinico-chemical examinations.

All F0 and F1 parental animals were assessed by gross pathology (including weight determinations of several organs) and subjected to an extensive histopathological examination, special attention being paid to the organs of the reproductive system.

Findings

The actual mean test substance uptake is given in Table 11.

Table 11. Mean test substance intake - 2-generation feeding study in Wistar rats (mg/kg bw/d)

Group/study phase		Dose level (ppm)			
		0 ppm (control)	10 ppm	25 ppm	250 ppm
F0 generation					
Males		0	0.9	2.4	24.1
Females	Pre-mating	0	1.0	2.6	25.8
Females (F1a litter)	Pregnancy	0	0.9	2.2	21.0
	Lactation*	0	1.4	3.7	31.1
Females (F1b litter)	Pregnancy	0	0.8	1.9	18.5
	Lactation *	0	1.2	3.1	30.4
F1 generation					
Males		0	0.8	1.9	20.1
Females	Pre-mating	0	0.9	2.2	22.0
Females (F2 litter)	Pregnancy	0	0.8	2.0	19.2
	Lactation *	0	1.3	3.1	29.2
Average F0 / F1 parental animals (pre-mating)		0	0.9	2.3	23

*days 0-14 post partum only

No parental and offspring toxicity or reproductive effects were observed at 10 and 25 ppm.
The following findings were observed and assessed to be test substance related at the 250 ppm dose level:

Parental animals:

Food consumption was reduced in F0 females during lactation of F1a litters, in F1 parental males especially at the beginning of the pre-mating period and in F1 parental females during pregnancy/lactation of the F2 litter. There was no effect on body weight and body weight gain in F0 parental animals while body weights were clearly reduced in F1 males and their body weight gain was impaired.

Time to mating was longer than 4 days (duration of a normal oestrous cycle) for three F0 (F1a litters) and four F1 mating pairs which may indicate irregularities of the oestrous cycle. In addition, male and female fertility indices were somewhat reduced; however, when F1a, F1b and additional matings with untreated partners were considered in combination, fertility was proven for all F0 sires and dams.

Vaginal haemorrhages were noted in six F0 dams pregnant with the F1a litter. Two of these dams were unable to deliver and died shortly after the expected delivery date; another dam died shortly after becoming pregnant with the F1b litter. Vaginal haemorrhage was also noted in one F1 dam which could not deliver pups after prolonged pregnancy. Prolonged duration of pregnancy (≥ 23 days) was noted in several F0 and F1 parental animals. However, at lower doses, especially after the F1b mating, epoxiconazole appeared to increase the variability of the duration of pregnancy as a number of unusually early deliveries (pregnancy day 20 or 21) were observed in the 10 and 25 ppm groups. At 250 ppm, the gestation index was reduced due to a high number of dams delivering stillborn pups or entirely dead litters (F1a litter). In F1 dams no complete litter losses at birth were observed, however, the number of dams delivering stillborn pups was increased. Due to the number of pups dying during parturition the number of live offspring/dam was moderately reduced in F1a and slightly reduced in F1b litters.

There were no test substance related effects with respect to clinical chemistry of F0 and F1 parental animals. Pathology examination revealed an increase of absolute and relative liver weights in F1 females without a histopathological correlate and a decreased fatty change in the liver of F1 males. Absolute and relative adrenal weights were reduced in F1 males. No findings were noted in the F0 generation.

Table 12. Parental findings (F0 and F1 animals)

Parameter and/or group affected	Dose level (ppm)			
	0 (control)	10	25	250
Food consumption	-	-	-	Reduced in F0 females during lactation of F1a litters; F1 males especially at the beginning of pre-mating period; F1 females during pregnancy/lactation
Body weight (g) of F1 males[$], week 25	555	557	554	493**
Body weight gain (g) of F1 males[$], weeks 0-25	363	362	365	322*
Clinical findings				
F0	-	-	-	Vaginal haemorrhages during pregnancy for the F1a litter in 6 F0 dams; 2 of these died with severe dystocia. One dam died during pregnancy for F1b litter
F1	-	-	-	Vaginal haemorrhage in one F1 dam which did not deliver pups after prolonged pregnancy
Precoital interval >4 days				
F1a	0	0	0	3
F1b	0	0	1	0
F2	0	0	0	4
Fertile matings				
F1a	24	24	24	22
F1b	24	25	23	18
F2	25	22	25	21
Pregnancy > 22 days (23 or 24 days)				
F1a	0	0	2	9*
F1b	3	1	4	3
F2	2	0	2	6*
Pregnancy < 22 days (20 or 21 days)				
F1a	0	0	2	0
F1b	1	4	5	2
F2	1	1	1	1
Gestation index				
F1a mating	100	100	100	73*
F1b mating	100	100	100	100
F2 mating	100	100	100	95
Pathology				

F1 parental animals	-	-	-	Increase of absolute and relative liver weights in females; decreased fatty change in the liver of males; decrease of absolute and relative adrenal weights in males

* $p \leq 0.05$ / ** $p \leq 0.01$ (Dunnett's Test; Fisher's Exact Test) $ (only F1 males affected)

Offspring:

The following findings were noted in pups:

At 10 and 25 ppm, a significant decrease in viability index in F2 pups was seen.

At 250 ppm:

There was a marked increase in the number of F1a, F1b and F2 pups which were stillborn and in the number of F2 pups which died or were cannibalised during the rearing period. Number of liveborn pups was significantly decreased. The viability index was lower for F1b and F2 litter. In addition, the lactation index was reduced for F2 pups. Pup mortality in the 10 ppm and the 25 ppm groups is not considered as substance-related since there is no clear dose-response relationship, and the values are within the range of historical control data as well as comparable to the concurrent control (F1a litter).

In three litters one pup each exhibited anasarca (generalised oedema) at birth. These pups were either stillborn or died early in the postnatal period. This finding, although occurring at a low incidence, was considered substance-related because of similar findings in a subsequent developmental toxicity study (Schneider *et al*. 2002).

Mean pup body weights at birth were similar to or higher than control data due to the extended intrauterine period. However, postnatal weight gain was slower than in controls and pup body weights lagged behind control values from postnatal day 7 onwards in both the F1 and the F2 litters. Two F1a litters showed a poor general state of health during the first days after birth. No clear effects on developmental landmarks were observed when the litter was considered as the relevant unit of comparison. Delays appeared more related to the shorter duration of pregnancy in individual litters than to an actual treatment effect on the pups and would have been more appropriately assessed by using post-coital time of development instead of postnatal timing.

Table 13. Pup findings (F1a/F1b and F2 pups)

Dose level (ppm)	0(control)	10 ppm	25 ppm	250 ppm
Stillborn pups (%)				
F1a	4.8	5.4	2.1	21**
F1b	4.6	2.3	4.5	12**
F2	2.0	0.7	2.5	18**
Litter death at birth (N)				
F1a	0	0	0	4
F1b	0	0	0	0
F2	0	0	0	0
Liveborn pups (Mean litter size)				
F1a	13.3	12.3	13.7	9.1**
F1b	13.8	13.7	13.0	12.2**
F2	11.7	12.9	10.8	10.9
Viability index				
F1a	90	90	92	93
F1b	95	94	93	90*
F2	98	93**	94**	82**
Lactation index				
F1a	99	99	99	99
F1b	100	99	99	99
F2	99	98	98	94**
Pup body weight gain day 4-21 (g)				
F1a	43.7	44.8	44.6	41.9
F1b	44.1	43.4	45.6	40.4
F2	41.1	40.0	40.6	35.5**
Clinical findings and abnormalities				
F1a: poor state of health (litters)	0	0	0	2
F2: anasarca (litters/pups)				3/3

* $p \leq 0.05$ / ** $p \leq 0.01$ (Dunnett's Test; Fisher's Exact Test)

Conclusion

The dietary administration of epoxiconazole to rats in doses of 250 ppm caused signs of systemic toxicity (e.g. decreased food consumption and increased liver weight) in the F0 females and in the F1 parental animals and their progeny. At this dose level some of the reproductive parameters relating to mating, impregnation and parturition were impaired, however, fertility of all parental animals could be established.

Ten and 25 ppm were tolerated by the parental animals. There was a significant decrease in viability index at 10 and 25 ppm in the F2 group, but not in the F1a group at any dose level and in the F1b group only at the highest dose level, which could indicate that effects on viability index may increase with the second generation. The finding of anasarca in three litters (one pup in each litter) was considered substance-related because of similar findings in a subsequent developmental toxicity study.

(Draft Assessment Report, Reference 2)

Report:	Schneider S. *et al*. 2002;TOX2002-2288 BAS 480 F - Prenatal developmental toxicity study in Wistar rats - Oral administration (gavage) BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF DocID 2002/1012810
Data taken from:	Draft Assessment Report, Reference 2.
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Guideline:	EEC 87/302 B, OECD 414, EPA/OPPTS 870.3700
Deviations:	The study exceeded test guidelines with respect to the scope of the examinations for the dams. Only 1 dose level was used instead of 3 dose levels.
Acceptability:	The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole, purity 94.7 %, batch 00-2046; purity 99.8 %, batch 40-96-1

Test animals: groups of 25 female Wistar CrlGlxBrlHan:WI rats, provided by Charles River, Sulzfeld, Germany

The aim of this study was to investigate maternal and developmental toxicity of epoxiconazole

- at a single dose level of 180 mg/kg bw which showed severe maternal and developmental toxicity effects in a previous study (see Hellwig and Hildebrand 1989)
- extending the scope of maternal toxicity determination as done in a previous study to haematology and clinico-chemical parameters and in addition to determination of hormones by radioimmunoassay
- comparing 2 batches of different purity

For this purpose 25 presumably pregnant female Wistar rats were treated by gavage from day 6 through day 19 post coitum (p.c.) with epoxiconazole doses of 0 (control treated with the vehicle 0.5 % aqueous carboxymethyl cellulose in double distilled water), or 180 mg/kg bw of epoxiconazole batch #1 (94.7 % active ingredient) or batch #2 (99.8 % active ingredient) in a constant dosing volume of 10 mL/kg bw.

Food consumption and body weights were recorded regularly throughout the study period. The animals' state of health was checked daily. On day 20 p.c., all females were sacrificed and haematological, clinico-chemical parameters and hormone levels were determined. The dams were assessed by gross pathology including weight determination of the unopened uterus and placenta. For each dam the number corpora lutea and distribution/number of implantation sites were determined. The foetuses were removed from the uterus, sexed, weighed and subsequently investigated for any external findings. Thereafter about half of them were examined for soft tissue findings and the remaining foetuses for skeletal (including cartilage) changes.

Table 14. Scope of haematological/clinical chemical and hormone parameters – developmental toxicity gavage study in Wistar rats

Haematological parameters:	Clotting analysis
Leukocytes	Prothrombine time
Erythrocytes	Clinical chemical parameters:
Haemoglobin	Alanine aminotransferase
Haematocrit	Aspartate aminotransferase
Mean corpuscular volume	Alkaline phosphatase
Mean corpuscular haemoglobin	Serum-gamma-glutamyltransferase
Mean corpuscular haemoglobin concentration	Sodium
Platelets	Potassium
Differential blood count	Chloride
Hormone determination:	Inorganic phosphate
Estradiol	Calcium
Corticosterone	Urea
Progesterone	Creatinine
Aldosterone	Glucose
Testosterone	Total bilirubin
Total triiodothyronine (T3)	Total protein
Total thyroxine (T4)	Albumin
Adrenocorticotropic hormone (ACTH)	Globulins
Luteinizing hormone (LH)	Triglycerides
Follicle stimulating hormone (FSH)	Cholesterol
Prolactin	Magnesium
Thyroid-stimulating hormone (TSH)	

Findings

Clinical parameters

Clinical symptoms observed were blood in bedding and/or vaginal haemorrhages in some dams in both groups (batch #1: 6 dams; batch #2: 2 dams; see Table 15). Piloerection was noted towards the end of the application period. Food consumption was reduced in both groups, but was seen earlier with batch #1. Body weight (day 20) and body weight gain (day 6-19) were affected with batch #1.

With both batches, body weight development and corrected body weight gain were significantly affected, and no difference in severity between the two batches could be observed.

Table 15. Clinical findings developmental toxicity gavage study in Wistar rats

Parameter and/or group	Dose (mg/kg bw/d)		
	0 (control)	180 (batch #1)	180 (batch #2)
Mated females on study	25	25	25
Pregnant females on study	19	22	22
Mortality of dams	0	0	0
Clinical symptoms:			
Blood in bedding and/or vaginal haemorrhage	0	6	2
Piloerection	0	3	1
Food consumption day 6-19 (g/animal/d)	19.7	16.9 day 6-8: - 30 %** day 13-20: - 17 %** day 6-19 p.c.: - 14 %	18.5 day 15-20: - 17 %** day 6-19: - 6 %
Body weight day 20 (g)	263.4	244.7 * day 8.: - 4 % day 20: - 7 %	258.3
Body weight gain day 6-19 (g)	68.4	55.5*	68.5
Body weight development		day 6-8: weight loss** day 19-20: -31 %** day 6-19: - 19 %*	day 6-8: - 40 %* day 19-20: - 57 %** day 6-19: - 4 %
Corrected (net) body weight gain (g, % of control)	34.1 100%	18.6 ** 55 %	23.7 ** 70 %

* $p<0.05$ **$p<0.01$

Clinical chemistry/haematology/hormone analysis

Decreases in several red blood cell parameters (red blood cells, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration) were observed in epoxiconazole-treated dams. Red blood cells, haemoglobin and haematocrit were decreased with batch #1, but when the number of dams with values below the concurrent control range (number of dams affected) was compared there was no obvious difference between the batches. In addition, the number of platelets was significantly decreased and the clotting time was increased (n.s.) for both batches to a similar extent, considering both mean counts and number of affected animals.

Changes of several clinico-chemical parameters were seen, usually in both treated groups, decreases for alanine aminotransferase and alkaline phosphatase, an increase for aspartate aminotransferase, and decreases for protein content (total protein, albumin and globulins), potassium and magnesium. Inorganic phosphate and serum urea values were increased. For the majority of these parameters the changes were slightly more pronounced for batch #1 with the higher impurities.

Estradiol, progesterone and prolactin values were markedly depressed with both batches, while LH was increased (n.s. with batch #2).

Table 16. Clinical chemistry/haematology – developmental toxicity gavage study in Wistar rats (changes given in % of control)

Parameter	Dose level (mg/kg bw/d)				
	0 (control)	180 (batch #1)	180 (batch #2)	180 (batch #1)	180 (batch #2)
Haematology				Dams outside control range	
Red blood cells (tera/L)	5.53	4.27* (-23 %)	5.09 (-8 %)	4/8	3/8
Haemoglobin (mmol/L)	6.8	5.1* (-24 %)	6.1 (-9 %)	4/8	4/8
Haematocrit (L/L)	0.303	0.235* (-22 %)	0.283 (-6 %)	5/8	3/8
Mean corpuscular haemoglobin concentration (mmol/L)	22.30	21.39 (-4 %)	21.72 (-3 %)	2/8	2/8
Platelets (giga/L)	838	481** (-43%)	549** (-34 %)	7/8	6/8
Clotting time (seconds)	22.3	24.1 (+8 %)	23.9 (+7 %)	0/8	0/8
Clinical chemistry				Dams outside control range	
Alanine aminotransferase (µkat/L)	0.85	0.79 (-7 %)	0.63** (-26 %)	2/8	6/8
Aspartate aminotransferase (µkat/L)	1.48	2.67* (+81 %)	2.05** (+38 %)	3/8	1/8
Alkaline phosphatase (µkat/L)	5.10	2.73** (-46 %)	4.60 (-10 %)	4/8	2/8
Total protein (g/L)	67.22	47.81 (-29 %)	56.42 (-16 %)	5/8	6/8
Albumin (g/L)	35.25	28.62* (-19 %)	31.90 (-10 %)	5/8	4/8
Globulin (g/L)	31.97	19.19** (-40 %)	24.52** (-23 %)	no data	no data
Potassium (mmol/L)	6.26	5.52** (-12 %)	5.77 (-8 %)	5/8	4/8
Magnesium (mmol/L)	0.99	0.86*(-13 %)	0.87 (-12 %)	4/8	4/8
Inorganic phosphate (mmol/L)	1.95	2.37** (+21 %)	2.16 (+11 %)	7/8	3/8
Urea (mmol/L)	7.33	8.20 (+12 %)	9.72* (+33 %)	1/8	2/8
Hormones				Dams outside control range	
Estradiol (pmol/L)	84.72	17.05** (-80 %)	18.22** (-79 %)	< 40: 15/16	< 40: 15/15
Progesterone (nmol/L)	197.89	86.81** (-56 %)	106.86** (-46 %)	< 60: 5/16	< 60: 3/15
LH (µg/L)	1.30	1.93* (+48 %)	1.75 (+34 %)	> 2.25: 5/16	> 2.25: 1/15
Prolactin (µg/L)	4.21	2.38** (-44 %)	1.35** (-68 %)	< 1.3: 8/16	< 1.3: 8/15

* $p < 0.05$, **$p < 0.01$ (Wilcoxon Test)

Necropsy findings

At necropsy, brownish amniotic fluid was observed in uterus in two females treated with batch #1 and two females treated with batch #2.

An enlarged spleen was observed in one female treated with batch #1 and one female treated with batch #2.

Caesarean section parameters

The total resorption rate, and especially late resorptions, and the number of dams losing more than 2 implants was significantly and similarly increased for both batches and corresponds to a post-implantation loss of about 40 - 60 % in the test groups versus 10 % in the control. In addition, placental weights were increased with both batches, but more pronounced with batch #1. The mean number of live foetuses per litter was significantly decreased in both test groups. Pre-implantation loss and implantation sites remained unaffected by epoxiconazole treatment.

Table 17. Data at caesarean section/foetal examination prenatal toxicity gavage study in Wistar rats

Parameter	Dose level (mg/kg bw/d)		
	0 (control)	180 (batch #1)	180 (batch #2)
Females mated	25	25	25
Pregnant dams	19	22	22
Litter death (resorption)	1	2	1
Premature birth	0	0	0
Dams with live foetuses	18	20	21
Gravid uterus weight (g)	47.5	41.0	50.4
Corpora lutea, CL (mean)	10.2	10.8	10.0
Implantation sites (mean)	9.1	9.1	9.4
Pre-impl. loss (> 2 CL)	4	8	0
Pre-implantation loss (mean %)	12.0	14.9	6.1
Post-impl. loss (> 2 implants)	0	18**	14**
Total resorptions (mean)	0.5	5.3**	4.0**
Early resorptions (mean)	0.4	0.8	0.5
Late resorptions (mean)	0.1	4.5**	3.5**
Live foetuses (mean)	9.1	4.2**	5.6**
% males	53	60	52
Placental weights in g (mean)	0.40	0.91**	0.76**
Foetal weights in g (mean)	3.5	3.3	3.4

* $p < 0.05$, ** $p < 0.01$

Foetal examination

There were no significant differences in sex distribution compared to controls. Placental weights were increased in both test groups by 128 % and 90 %, respectively, which could be related to the hormonal imbalance or could be prompted by an induction of placental metabolic activity. Foetal

weights were not significantly decreased (maximum 6 % when compared to control). In both treated groups a number of substance-related malformations and variations were found (see Table 18 and 19).

Table 18. Individual external foetal malformations prenatal toxicity gavage study in Wistar rats

Test group	No of dam – no. of foetus Sex*	Type of malformation
0 (control)	-	No malformations observed
1 (batch #1 : 180 mg/kg bw/d)	34 – 03 M	Anasarca, domed head
	35 – 03 F	Anasarca
	37 – 03 M	Cleft palate
	37 – 05 M	Cleft palate
	44 – 03 F	Anasarca
	44 – 04 M	Anasarca, malrotated limb
	45 – 05 M	Anasarca, domed head, macroglossia
2 (batch #2 : 180 mg/kg bw/d)	63 – 03 F	Anasarca
	68 – 08 M	Anasarca
	70 – 08 M	Cleft palate

*M – male , F - female

The most frequently observed external malformation was anasarca, a generalized oedema, which occurred in five batch #1 (statistically significantly increased) and two batch #2 foetuses (n.s.). It was associated with domed head in two batch #1 foetuses, from which one showed in addition a macroglossia. Moreover, cleft palate was recorded for two batch #1 foetuses and one foetus of batch #2. Soft tissue malformations that could be attributed to the test substance application were not observed. Both the foetuses that exhibited a domed cranium were assigned to skeletal examination. A diagnosis of hydrocephalus could therefore not be confirmed.

Significantly increased incidences of several skeletal malformations were found with both batches, but was more pronounced with batch #1 (see Table 19).

Table 19. Foetal skeletal malformations and variations (%) in the prenatal toxicity gavage study in Wistar rats

Findings (affected foetuses/litter mean %)	Test group 0, control	Test group 1: 180 mg/kg bw/d, batch #1	Test group 2: 180 mg/kg bw/d, batch #2	Historical control Mean % (range)
Deformed clavicle	0	4.3	0	Not present
Absent tuberositas deltoidea	0	52.5**	1.6	Not present
Small tuberositas deltoidea	0	31.6**	0	Not present
Supraoccipital hole(s)	49.0	92.5**	88.9**	24.2 (8.6-50.1)
Unossified hyoid	0	7.1*	0	Not present
Incomplete ossification of basisphenoid	6.7	50.6**	47.4**	9.2 (2.8 - 14.7)
Basioccipital hole(s)	0	15.8*	4.0	1.5 (0.0 - 3.6)
Incomplete ossification of lumbar arch	0	31.9**	11.8**	0.1 (0.0 - 0.8)
Unossified sternebra	5.0	45.5**	43.2**	9.7 (3.4 - 20.4)
Incomplete ossification of sternebra	49.2	68.9*	57.1	51.0 (41.3 - 60.0)
Misshapen sternebra ; normal cartilage	46.1	79.0**	76.3**	19.0 (7.7 - 49.7)
Unilateral ossification of sternebrae ; normal cartilage	1.4	12.3*	7.1	1.2 (0.0 - 2.6)
Bipartite ossification of sternebra ; normal cartilage	0	12.3*	1.6	0.6 (0.0 - 2.2)
Cervical rib ; cartilage not present	0.9	37.1**	13.3**	3.7 (0.7 - 7.5)
Cervical rib ; cartilage present	0	15.0*	4.0	Not present
Long supernumerary rib (14^{th})	4.7	43.3**	35.2**	2.6 (0.0 - 4.5)
Rudimentary 15^{th} rib	0	11.0*	0	Not present
Total foetal skeletal variations	98.9	100	100	92.4 (87.0 - 98.1)

* $p < 0.05$, **$p < 0.01$

Conclusion

The oral administration of epoxiconazole to pregnant Wistar rats caused impairments in food consumption and body weight, clear indications of hormonal imbalances, anaemia, influences on liver function and increased placental weights in treated dams. Effects were observed in both batches, but were somewhat more pronounced in batch #1. These findings were accompanied by overt developmental toxicity like a very high incidence of post-implantation loss and total resorptions. There were also distinct effects on foetal morphology in both batches, being more pronounced with batch #1. Compared to the dose-finding study, which was conducted with an epoxiconazole batch with 92.8 % purity, both batches in this study contained less impurities. Both induced a much higher post-implantation loss than was observed in the dose-finding study. In contrast, cleft palate, a high prevalence malformation in the dose-finding study, was only observed in one litter each. This is, however, not thought to be attributed to different concentrations of impurities, but to the extension of treatment duration from days 6 - 15 of pregnancy to days 6 - 19 which appears to have been responsible for the increased embryo/foetolethality. Embryos that are more sensitive to the teratogenic action of a substance can be also expected to be more susceptible

to embryolethality than their more resistant littermates and thus would be eliminated preferentially by resorption before their aberrant development could be detected. The extended treatment period identified effects of epoxiconazole on bone formation during the foetal period.

(Draft Assessment Report, Reference 2)

Report: Taxvig C *et al*. 2007, Toxicological Sciences, 100(2), 464-473. Endocrine disrupting activities *in vivo* of the fungicides tebuconazole and epoxiconazole.

GLP: Not stated

Guideline: No

Acceptability: Acceptable/Non-guideline

New study not included in the Draft Assessment Report.

For further details, Taxvig *et al*, 2007.

Material and methods

Test material: Pure epoxiconazole (99%) from Dr Ehrenstorfer (Augsburg, Germany). As vehicle, corn oil from Bie & Berntsen (Rødovre, Denmark) was used.

Test animals: 112 young adult time-mated Wistar rats (HanTac: WH, Taconic M&B, Ejby, Denmark), which were supplied on GD 3 were used for the entire study, including the testing with tebuconazole. The animals were distributed in pairs and housed under standard conditions. The day after arrival (GD4), animals were weighed and assigned to five groups (including two groups that were administered tebuconazole; results will not be presented here). The three groups that were included in the epoxiconazole testing consisted of 24 animals each (n=72), with similar weight distribution. Epoxiconazole was administered by oral gavage at doses of 0 (control), 15 or 50 mg/kg bw from GD 7 until PND 16. The doses were based on literature survey. Of the time-mated animals, 19, 19 and 21 in the control, 15 and 50 mg/kg bw groups, respectively, were pregnant.

Females were observed for clinical signs of toxicity daily, and body weights were recorded on GD 4 and then daily during the dosing period. Maternal body weight gain from GD 7 to 21 and from GD 1 to PND 1 was calculated.

At the beginning of the study, some dams in each group were selected for Caesarian section on GD 21. Additional sections on four animals in the highest dose group had to be performed on GD 24-25 because of dystocia, and the final number of animals selected for Caesarian section was then 6, 9 and 14 in the control, 15 and 50 mg/kg group, respectively. The uteri were taken out and the number of live foetuses, location in the uterus, resorptions and implantations were registered. Body weight, sex and any anomalies of the offspring were recorded. Trunk blood was collected immediately after decapacitation from all foetuses for hormone analysis and one pool per litter was made for all male and female foetuses, respectively.

At the day of delivery (PND 0), time of birth, weights of dams and individual pups were recorded, and the pups were counted, sexed and checked for anomalies. In all live pups anogenital distance (AGD) was measured using a stereomicroscope. On PND 13, all male and female pups were weighed and examined for the presence of areolas/nipples. The external genitals were inspected

(blinded to the observer) on PND 16 in all males from all litters. From the control and 15 mg/kg group, 1 – 2 males and females/litter were saved for further studies, and the rest of the pups were sacrificed on PND 16 and trunk blood collected and pooled to one male and one female sample/litter. Body weights were recorded and in 1 – 2 males per litter, and several organs (liver, kidneys, adrenals, testes, epididymes, seminal vesicles, ventral prostate, bulbourethral glands, and levator ani/bulbocavernosus muscles (LABC)) were excised and weighed. From 1 – 2 males/litter, the right or left testes were fixed and in one male/litter, organs (ventral prostate, seminal vesicles, epididymes, thyroids and adrenals) were fixed in formalin and embedded in paraffin. All fixed organs were examined by light microscopy. In 1 – 2 females/litter, body weights were recorded, and in one female/litter the thyroids, ovaries and uterus were excised and weighed.

At GD 21 or PND 16, levels of testosterone and progesterone were analysed in serum from the pups. Steroid hormones and 17α-hydroxyprogesterone levels were analysed in testis and estradiol was analysed in ovaries. Foetal testes were taken out on GD 21 from 1-3 males/litter for determination of hormone levels or *ex vivo* testosterone production.

Findings

The highest dose of epoxiconazole increased gestational length, loss of foetuses and postnatal deaths of the pups, induced dystocia and a high frequency of stillbirths, leading to a reduction in live litter size. Many of the dead foetuses had died very late in the gestation period (27/128), and this was not seen in the control group. No statistically significant toxic effects on foetuses or mothers were seen with the low dose. On GD 21, a decrease in foetal weight in both sexes was seen in the highest dose group.

Anogenital distance was increased both in female foetuses at GD 21 and in newborn female offspring. In male foetuses, a significantly increased AGD was seen when dividing the AGD with the cubic-root of the body weight. In male offspring, no effects on AGD were seen. Before dividing AGD with the cubic-root of body weight, AGD was significantly increased in the low dose group, but after division no change could be detected.

No effects of epoxiconazole on the measured hormone levels in foetuses on GD 21 were found. In plasma from treated dams, an increase in progesterone level (7-fold) and an increase in testosterone (2-fold) were seen. A tendency towards lowered estradiol levels in female pups on PND 16 was seen, but this was not statistically significant. There was also a tendency (n.s.) towards a decrease in testosterone in males at PND 16.

On PND 16, a tendency towards increased liver weight was seen in the highest dose group, and there was also a tendency towards an increased weight of all male reproductive organs, without any effects on body weight. However, only few animals were analysed and the results should be interpreted carefully. No histopathological effects on the testes (except for one Sertoli cell only-testis in the lowest dose group) were found. No effects on female reproductive organ weights were seen.

Table 20. Pregnancy and Litter Data (as in Taxvig *et al*, 2007)

	Control	Epo-15	Epo-50
Dams and litters			
No. of dams (viable litters)	$N = 13$ (13)	$N = 10$ (9)	$N = 7$ (1–2)[a]
Maternal weight gain GD 7–21	85.38 ± 11.9	87.70 ± 16.1	92.14 ± 14.0
Maternal weight gain GD 7–PND 1	20.62 ± 7.2	18.11 ± 6.7	15.00 ± 8.5
Body weight gain PND 1–13	7.53 ± 16.3	−6.89 ± 24.8	*20*
Gestation length (days)	22.46 ± 0.5	22.67 ± 0.7	**23.71 ± 0.8****
% Postimplantation loss	6.55 ± 5.1	16.01 ± 30.0	**34.25 ± 18.2***
% Perinatal loss	9.67 ± 8.0	18.21 ± 30.0	**88.78 ± 29.7****
Litter size	11.15 ± 1.7	9.90 ± 4.1	8.67 ± 3.1
Born alive per litter	10.92 ± 1.7	9.80 ± 4.0	**4.33 ± 5.9****
Born dead per litter	0.23 ± 0.4	0.10 ± 0.3	**4.33 ± 2.9****
% Postnatal death	3.39 ± 5.6	2.78 ± 5.9	**69.44 ± 52.9****
% Males	44.76 ± 17.6	45.45 ± 13.1	34.72 ± 33.4
Offspring (data from viable litters)			
Birth weight (g)	5.53 ± 0.3	**6.21 ± 0.6****	6.36 ± 0.3
Body weight PND 13 (g)	23.25 ± 2.6	21.48 ± 4.8	*23.4*
Male AGD (mm)	3.41 ± 0.2	**3.65 ± 0.2***	3.41 ± 0.3
Male AGD per cubic root body weight	1.92 ± 0.1	1.96 ± 0.1	1.83 ± 0.2
Female AGD (mm)	1.72 ± 0.1	**1.95 ± 0.2****	*1.71*
Female AGD per cubic root body weight	0.98 ± 0.03	**1.08 ± 0.1***	*0.96*
No. areolas males	2.08 ± 0.6	2.53 ± 1.1	*3.38*
No. areolas females	12.5 ± 0.4[c]	12.32 ± 0.2	*12*
GD 21 cesarean section			
No. of dams	$N = 6$	$N = 9$	$N = 14 + 4$[a]
Maternal body weight (g)	307.17 ± 22.4	287.33 ± 29.8	285.73 ± 17.7
Adjusted body weight (g)	232.70 ± 14.9	231.49 ± 15.9	223.13 ± 21.0
No. of implantations	12.50 ± 2.1	10.22 ± 4.3	12.06 ± 2.4
No. of fetuses	11.67 ± 2.1	9.11 ± 4.9	9.00 ± 4.1
% Postimplantation loss	6.45 ± 7.9	20.87 ± 33.5	**28.14 ± 26.9***
% Late resorptions	1.28 ± 3.1	13.89 ± 33.3	**24.88 ± 27.3***
% Very late resorptions	0.0 ± 0.0	4.16 ± 11.8	**15.12 ± 24.0***
% Males	56.01 ± 17.2	46.41 ± 18.8	53.57 ± 25.3
Fetal weight male (g)	4.45 ± 0.3	3.98 ± 0.9	**3.79 ± 0.7***
Fetal weight female (g)	4.18 ± 0.4	3.8 ± 0.8	**3.54 ± 0.7***
No. of litters for AGD[b]	$N = 3$	$N = 6$	$N = 9$–10
Male AGD (mm)	3.39 ± 0.3	3.54 ± 0.1	3.40 ± 0.1
Male AGD per cubic root body weight	2.08 ± 0.1	**2.31 ± 0.1***	2.25 ± 0.1
Female AGD (mm)	1.65 ± 0.1	**1.91 ± 0.3****	**1.92 ± 0.1****
Female AGD per cubic root body weight	1.04 ± 0.1	**1.28 ± 0.2***	**1.29 ± 0.1****

Data represent group means based on litter means ± SD. Epo-15 and Epo-50 = 15 and 50 mg/kg bw/day epoxiconazole. Values written in italic are from only one dam per litter and therefore no SD are shown. Values shown in bold are statistically significantly different compared to control, * $p < 0.0.5$ and ** $p < 0.01$. [a]Because of problems with parturition caesarean section (CS; GD 23–25) was performed on four additional dams in Epo-50. These data were included in the analysis of GD 21 CS data. [b]AGDs were only measured in the second set of animals. [c]Some female pups had 13 nipples.

Conclusion

Epoxiconazole had a marked foetotoxic effect and the dams in the highest dose group were in general unable to deliver their pups. Only two out of 7 litters were born normally, and the rest was

instead included in the Caesarian section group. In female offspring, AGD was increased, indicating a virilising effect. There were indications of an effect on AGD in males, but the effects were not consistent between foetuses and pups or between the AGD and the anogenital index (i.e. the anogenital distance adjusted for weight differences). The increased gestational length is probably due to the increased levels of progesterone that was seen in the dams, and this increase is probably also involved in the virilising effect seen in the female offspring.

The study shows that epoxiconazole has endocrine disrupting effects *in vivo*, resulting in effects on both dams and offspring, and in particular female foetuses and offspring.

Rabbit

Report: Hellwig J., Hildebrand B. 1990(a);TOX2003-1851
Study of the prenatal toxicity of Reg. No. 205 259 in rabbits after oral administration (gavage)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 90/0213

Data taken from: Draft Assessment Report, Reference 2.

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Guideline: OECD 414, EPA 83-3, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole, purity 93.2 %, batch CP 2431

Test animals: groups of 15 female Himalayan rabbits, provided by Karl Thomae, Biberach, Germany

Epoxiconazole was administered to artificially inseminated rabbits from day 7 through 19 post insemination (p.i.) in 0.5 % aqueous carboxymethyl cellulose (CMC) preparation. Doses selected were 0 (control), 5; 20 and 80 mg epoxiconazole/kg bw in a constant volume of 10 mL/kg bw. The test substance was prepared daily.

Body weight and food consumption was monitored throughout the study. The animals were examined daily for mortality and clinical symptoms. All surviving animals were sacrificed on day 29 p.i. and assessed by gross pathology. Uterine contents, position of the foetuses and number of corpora lutea were examined and placentas were weighed. The foetuses were dissected from the uterus, sexed, weighed and further examined for external, soft tissue and skeletal changes.

Findings

Clinical findings

Food consumption was reduced at 80 mg/kg bw/d and 20 mg/kg bw/d during the treatment period. Although statistically significantly affected also at the low dose this effect is not considered to be of biological relevance when body weight data, food spillage possibilities and dose response are taken into account. Body weight gain was significantly reduced during the treatment period, especially during days 11 to 14 p.i., at the high dose level. A smaller reduction (n.s.) was also noted at the mid dose level. One doe in the mid and one in the high dose group had blood in the bedding on day 16 or 20 - 21 p.i, respectively. The animal in the mid dose group also showed reduced defecation on days 18 - 26 p.i. Abortion was noted in one doe of the mid dose level at day 18 p.i.; this animal was sacrificed prematurely. No clinical findings were noted at the low dose level of 5 mg/kg bw/d.

Table 22. Maternal data - prenatal gavage toxicity study in Himalayan rabbits

Parameter	Dose level (mg/kg bw/d)			
	0	5	20	80
Mated females on study	15	15	15	15
Pregnant females on study	14	14	15	13
Mortality of dams	0	0	1 (aborted)	0
Clinical symptoms: blood in bedding	0	0	1	1
Food consumption day 7 - 19 (g)	90.6	76.4**	79.0*	79.1*
Body weight day 29 (g)	2,822	2,814	2,715	2,730
Body weight gain days 11 - 14 (g)	36	37	20	5.5*
Corrected (net)body weight change from day 6 (g)	- 112	- 161	- 131	- 125
Uterus weight (g)	310	382	281	242

* $p < 0.05$; **$p < 0.01$

Caesarean section/foetal evaluation

Embryotoxicity was observed in form of marked increased post-implantation loss due to an increased resorption rate at the high dose level of 80 mg/kg bw where 3 does had no viable foetuses. No other embryotoxic/foetotoxic effects were noted at any dose level. There was no increase in number of variations or malformations which could be attributed to the administration of epoxiconazole.

Table 23. Data at caesarean section/foetal examination - prenatal toxicity gavage study in Himalayan rabbits

Parameter	Dose level (mg/kg bw/d)			
	0 (control)	5	20	80
Females mated	15	15	15	15
Pregnant dams	14	14	15	13
Aborted	0	0	1	0
Premature birth	0	0	0	0
Litter death	0	0	0	3
Dams with viable foetuses	14	14	14	10
Corpora lutea, CL (mean)	7.5	8.7*	7.4	8.4
Implantation sites (mean)	6.2	6.8	5.6	7.5
Preimpl. loss (> 2 CL)	2	5	5	0
Preimplantation loss (mean %)	16.2	21.7	23.9	10.0
Postimpl. loss (> 2 implants)	1	0	0	6
Post-implantation loss (mean %)	13.6	2.3	12.2	43.0**
Total resorptions (mean)	0.9	0.1	0.5	3.3**
Early resorptions (mean)	0.6	0.1	0.4	3.2**
Late resorptions (meal)	0.2	0.0	0.1	0.2
Dead foetuses	0	0	0	0
Live foetuses (mean)	5.4	6.6	5.1	5.5
Placental weights in g (mean)	4.8	4.6	4.6	4.4
Foetal weights in g (mean)	41.9	42.3	39.5	41.0
Total malformations: foetal incidence n (%)	2 (2.7)	2 (2.2)	0 (0.0)	0 (0.0)
Total variations: Foetal incidence n (%)	1 (1.3)	1 (1.1)	1 (1.4)	2 (1.6)
Total retardations: Foetal incidence n (%)	45 (60.0)	51 (45.4)	27 (38.0)*	22 (40.0)**

* $p < 0.05$; **$p < 0.01$

Conclusion

Epoxiconazole caused reduced food consumption in treated dams at all dose levels. In isolated cases blood in the bedding (1 doe at the high and mid dose level, the latter showing also reduced defecation) and abortion (1 doe at the mid dose level) were noted. At the high dose level, a marked increase in the incidence of post-implantation loss together with an increase in the number of early and total resorptions was seen.

(Draft Assessment Report, Reference 2)

5.9.2.2 Dermal application

Report: Hellwig J., Hildebrand B. 1993;TOX2003-1850
Study of the prenatal toxicity of Reg. No. 205 259 in rats after dermal application
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF RegDoc# 1993/10151

Data taken from: Draft Assessment Report, Reference 2.

GLP: Yes (laboratory certified by Ministerium fuer Umwelt und Gesundheit, Postfach 3160, 6500 Mainz)

Guideline: EEC 87/302, EEC 67/548, OECD 414, EPA 83-3, JMAFF

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole, purity 93.2 %, batch CP 2431

Test animals: groups of 25 female Wistar Chbb:THOM (SPF) rats, provided by Karl Thomae, Biberach, Germany

Dams were treated dermally from day 6 through day 15 post coitum (p.c.) with epoxiconazole doses of 0 (control treated with the vehicle aqueous 0.5 % carboxymethyl cellulose); 100; 400 and 1000 mg/kg bw at a constant dosing volume of 2 mL/kg bw. The test substance preparation was administered to the intact, shaven dorsal skin for 6 hours/treatment day under semi-occlusive conditions.

Food consumption and body weights were recorded regularly throughout the study period. The state of health was checked daily. Special attention was given to the treated skin, which was examined daily prior to the compound application and after the 6-hour treatment period during days 6 - 15 p.c. On day 20 p.c., all females were sacrificed. The weights of the liver, kidneys and the uterus (before it was opened) were determined and the dams were assessed by gross pathology. The foetuses were dissected from the uterus, sexed, weighed and further investigated for external, soft tissue and/or skeletal changes. The weights of the placenta were determined.

Findings

There were no effects on food consumption, body weight, body weight gain at all dose levels including 1000 mg/kg bw/d, which is the highest dose recommended for this study type.

At the highest dose level the following findings were seen:

- statistically significantly increased placental weights
- one foetus with a cleft palate, another with short tail

- increased number of foetuses with skeletal variations (rudimentary cervical and/or accessory 14th rib(s)) (an increase was also seen at 400 mg/kg bw, n.s.)

Table 21. Data at caesarean section/foetal examination prenatal toxicity dermal study in Wistar rats

Parameter	Dose level (mg/kg bw/d)			
	0	100	400	1000
Females mated	25	25	25	25
Pregnant dams	23	24	24	25
Aborted	0	0	0	0
Premature birth	0	0	0	0
Litter death	0	0	0	0
Female mortality	0	0	0	0
Corpora lutea, CL (mean)	15.5	15.3	14.8	15.4
Implantation sites (mean)	15.0	14.5	13.7	14.9
Preimpl. loss (> 2 CL)	1	2	2	1
Preimplantation loss (mean %)	3.3	5.0	7.1	3.2
Postimpl. loss (> 2 implants)	4	2	1	3
Post-implantation loss (mean %)	8.8	6.7	5.9	8.3
Total resorptions (mean)	1.3	1.0	0.8	1.2
Early resorptions (mean)	1.0	0.8	0.7	1.1
Late resorptions (mean)	0.3	0.3	0.1	0.2
Dead foetuses	0	0	0	0
Live foetuses (mean)	13.6	13.5	12.9	13.7
Placental weights in g (mean)	0.44	0.43	0.46	0.50**
Foetal weights in g (mean)	3.8	3.8	3.9	3.9
Cervical ribs (litters/foetuses)	1/2	2/2	5/8	9/12
14th ribs (litters/foetuses)	1/1	1/1	4/6	7/10

* $p < 0.05$; ** $p < 0.01$

Conclusion

Dermal administration of 1000 mg/kg epoxiconazole did not result in any effects on maternal food consumption and weight. There was a significant increase of placental weight. Foetal effects noted at this dose level were an increase in the number of foetuses with skeletal variations (rudimentary cervical and/or accessory 14th rib(s). The effects are considered to be treatment-related as similar increases in rib number were consistently observed in oral studies with Wistar rats. In addition, one foetus with a cleft palate was noted at this dose level.

(Draft Assessment Report, Reference 2)

5.9.3 Human data

5.9.4 Other relevant information

5.9.4.1 Testing of endocrine disruptive properties

Report: Mellert W. 1992; TOX2003-1857 Interim report - Determination of hormone concentrations in Wistar rats treated with Reg. No. 205 259 BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 92/10715

and

Mellert W., Hildebrand B. 1999;TOX2003-1858; Amendment No. 1 to the interim report: Determination of hormone concentrations in Wistar rats treated with Reg. No. 205 259 BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep. unpublished BASF RegDoc# 1999/11334

Data taken from: Draft Assessment Report, Reference 2.

GLP: No

Guideline: There are no guidelines for this study type, but the study is considered scientifically valid.

Deviations: Individual data for body weights and hormone determinations are lacking. No data is presented on the analytical methods used for the hormone determinations.

Acceptability: The study is considered to be supplementary.

Material and Methods

Test material: Epoxiconazole; purity and batch not specified in the report.

Test animals: groups of 10 male and 20 female Wistar Chbb:THOM (SPF) rats, provided by Karl Thomae, Biberach, Germany

Epoxiconazole was administered to male and female Wistar rats via the diet or by gavage (0.5 % aqueous suspension in Tylose CB 3.000) at different dose levels for 4 - 6 days or for approximately 4 weeks. The study design is shown in Table 24.

Table 24. Study design for hormone investigations

Test group	Dose level	Number of animals			
		Males		Females*	
		4 days	4 weeks	4 – 6 days	4 weeks
0	0 ppm	10	10	20	20
1	1500 ppm	10	10	20	20
2a	3000 ppm	-	10	-	20
2b	200 mg/kg bw/d	10	-	20	-

* Female animals were sacrificed based on their oestrus status; if possible, 10 females were sacrificed in prooestrus and 10 females were sacrificed in dioestrus.

At the end of the respective administration periods the animals were sacrificed by decapitation and blood was taken for hormone determinations. It was not clear from the report, whether serum, plasma, or whole blood was used for hormone determinations. No information was given on the analytical method used.

Findings

Test substance intake is shown in Table 25.

Table 25. Test substance intake (mg/kg bw/d)

Test group	Dose level	Test substance intake (mg/kg bw/d)			
		Males		Females	
		4 days	4 weeks	4 – 6 days	4 weeks
1	1500 ppm	80	90	65	94
2a	3000 ppm	103	166	70	160
2b	200 mg/kg bw/d	200	-	200	-

There were no mortalities during the study. Females treated with 200 mg/kg bw/d showed a reduced general state of health at the end of the administration period.

Food consumption in the dietary studies was reduced for both males (during the first two weeks of treatment) and females (during the whole study period) in the 3000 ppm group. In males treated with 1500 ppm, food consumption was reduced during the first week and in females receiving 1500 ppm food consumption was reduced during the two weeks of treatment.

Body weight in the dietary studies was reduced in the 3000 ppm group for both males (-8 % at the end of the treatment period) and females (-10 % after 14 days of treatment). Animals receiving an oral dose of 200 mg/kg bw/d lost body weight resulting in a reduction of 11.5 % (males) and 10.2 % (females) as compared to the controls within three days of treatment.

In treated females of all dose groups, oestrous cycles were prolonged shortly after the beginning of treatment. The increased duration of the oestrous cycle could not be attributed to any particular stage of the oestrous cycle, as most females showed an irregular pattern.

Table 26. Hormone determinations – males, 4 days treatment

Hormone	Unit	0 ppm	1500 ppm	200 mg/kg bw/d
Dehydroepiandrosterone	ng/mL	0.56 ± 0.18	0.66 ± 0.19	0.49 ± 0.16
Androstenedione	ng/mL	0.62 ± 0.36	0.83 ± 0.33	1.1 ± 0.33*
Testosterone	ng/mL	2.3 ± 0.9	4.9 ± 2.2**	4.3 ± 3.2
Estradiol	pg/mL	7.4 ± 2.5	5.4 ± 3.5	4.3 ± 2.9
LH	ng/mL	2.2 ± 0.5	2.2 ± 0.3	2.3 ± 0.2
FSH	ng/mL	38 ± 14	63 ± 38	72 ± 55
Prolactin	ng/mL	11 ± 11	13 ± 8.5	1.7 ± 1.4
Corticosterone	pg/mL	162 ± 122	165 ± 62	189 ± 120
Aldosterone#	pg/mL	57 ± 10	144 ± 52	81 ± 55
ACTH	pg/mL	130 ± 106	52 ± 15*	112 ± 68

*p < 0.05, ** p < 0.01, #: no statistical evaluation possible due to low number of samples

Table 27. Hormone determinations – males, 4 weeks treatment

Hormone	Unit	0 ppm	1500 ppm	3000 ppm
Dehydroepiandrosterone	ng/mL	0.80 ± 0.21	0.93 ± 0.38	0.75 ± 0.22
Androstenedione	ng/mL	0.46 ± 0.10	0.76 ± 0.37*	0.78 ± 0.38*
Testosterone	ng/mL	2.9 ± 1.5	4.0 ± 2.1	3.6 ± 1.3
Estradiol	pg/mL	5.1 ± 3.2	5.3 ± 3.4	3.4 ± 2.2
LH	ng/mL	2.3 ± 0.2	2.4 ± 0.3	2.7 ± 0.7
FSH	ng/mL	29 ± 5.3	67 ± 47*	60 ± 16**
Prolactin	ng/mL	4.8 ± 2.6	7.7 ± 7.2	2.1 ± 1.2*
Corticosterone	pg/mL	160 ± 58	114 ± 38	94 ± 19*
Aldosterone	pg/mL	169 ± 88	141 ± 68	126 ± 49
ACTH	pg/mL	55 ± 23	55 ± 10	70 ± 36

*p < 0.05, ** p < 0.01

After 4 days of treatment some hormone changes were detectable in males although it appears that variability may have precluded the detection of any change in FSH and prolactin. An increase in testosterone levels, as well as a decrease estradiol, was seen although not being statistically significant (see Table 26). Only androstenedione showed a dose-related increase, being statistically significant at the highest dose level.

After 4 weeks of treatment, several hormones showed a significant difference (see Table 27), or a trend, which was considered to be test substance-related.

Table 28 shows further hormone alterations due to the administration of the test substance to males at 3000 ppm for 4 weeks.

Table 28. Hormone level alterations in males after a 4-week treatment with 3000 ppm (% of control)

Testosterone	124 %
Androstenedione	170 % *
Estradiol	67 %
FSH	207 % **
Corticosterone	59 % *
Aldosterone	75 %
ACTH	127 %

*p < 0.05 ; ** p < 0.01

In females, changes in hormone values during dioestrus after 4 days of treatment can only be evaluated for the group fed 1500 ppm since in many cases the number of data points at 200 mg/kg were too few for statistical evaluation. After 4 days treatment (see Table 29.), significantly increase levels of dehydroepiandrosterone, androstendione, LH and FSH were seen together with a decrease in estradiol and corticosterone, although the latter not being statistically significant. After 4 weeks, changes in the same hormones could be seen, the increase in estradiol levels being statistically significant at the highest dose level (not measured after 4 days) In addition, an increase in ACTH was seen at 3000 ppm.

Table 29. Hormone determinations in females - dioestrus

Hormone	Unit	After 4 days		After 4 weeks		
		0 ppm	1500 ppm	0 ppm	1500 ppm	3000 ppm
Dehydroepi-androsterone	ng/mL	0.74 ± 0.39	2.2 ± 0.5***	0.48 ± 0.18	1.5 ± 0.48**	1.3 ± 0.32***
Androstenedione	ng/mL	0.52 ± 0.15	1.8 ± 0.79***	0.32 ± 0.12	1.5 ± 0.52***	1.8 ± 0.45***
Estradiol	pg/mL	9.7 ± 7	3.6 ± 4.4	22 ± 18	4.5 ± 4.4	3.1 ± 3.5**
LH	ng/mL	2.2 ± 0.3	4.8 ± 1.9**	2.1 ± 0.3	3.0 ± 0.7**	4.7 ± 1.6***
FSH	ng/mL	14 ± 0.5	18 ± 3.4**	14 ± 1.0	16 ± 1.2*	18 ± 2.4**
Prolactin	ng/mL	3.5 ± 4.3	3.8 ± 4.2	1.9 ± 1.3	2.1 ± 1.9	2.2 ± 1.4
Corticosterone	pg/mL	425 ± 173	254 ± 204	303 ± 188	256 ± 161	253 ± 117
Aldosterone	pg/mL	--	--	386 ± 299	240 ± 134	144 ± 77
ACTH	pg/mL	98 ± 29	56 ± 23	65 ± 21	93 ± 55	201 ± 116*

*p < 0.05, ** p < 0.01, *** p < 0.001

During prooestrus (see Table 30), changes in the same hormones as during dioestrus was seen, but the decrease in estradiol was more pronounced, being statistically significant at all dose levels after both 4 days and 4 weeks of treatment. There was also a decrease in prolactin not seen at dioestrus.

Table 30. Hormone determinations in females - prooestrus

Hormone	Unit	After 4 days		After 4 weeks		
		0 ppm	1500 ppm	0 ppm	1500 ppm	3000 ppm
Dehydroepi-androsterone	ng/mL	1.2 ± 1.9	2.0 ± 0.69**	1.0 ± 0.23	2.1 ± 0.51***	1.5 ± 0.55*
Androstenedione	ng/mL	0.95 ± 0.18	2.3 ± 1.2**	0.95 ± 0.20	3.5 ± 1.4***	2.2 ± 1.7
Estradiol	pg/mL	45 ± 9.3	6.2 ± 5.4***	39 ± 6.8	19 ± 7.6***	8.1 ± 5.3***
LH	ng/mL	7.2 ± 7.1	5.3 ± 5.4	5.9 ± 3.3	10.0 ± 8.6	2.9 ± 0.4
FSH	ng/mL	18 ± 6.4	17 ± 4.0	17 ± 2.0	21 ± 5.0*	18 ± 1.5
Prolactin	ng/mL	129 ± 33	54 ± 59**	107 ± 39	92 ± 32*	4.7 ± 6.0***
Corticosterone	pg/mL	599 ± 264	393 ± 139	447 ± 170	328 ± 137*	160 ± 110***
Aldosterone	pg/mL	--	--	518 ± 277	317 ± 149*	81 ± 63***
ACTH	pg/mL	147 ± 78	64 ± 13*	77 ± 24	83 ± 41	112 ± 28*

*p < 0.05, ** p < 0.01, *** p < 0.001

Table 31 shows further hormone alterations due to the administration of the test substance to females at 3000 ppm for 4 weeks.

Table 31. Changes of hormone values in high dose females (% of control value)

Hormone	Dioestrus	Prooestrus
Dehydroepiandrosterone	270***	150**
Androstenedione	470***	232
Estradiol	14*	21**
LH	224***	117
FSH	129**	106
Prolactin	105	44**
Corticosterone	83	36***
Aldosterone	37	15***
ACTH	309*	145*

*p < 0.05, ** p < 0.01, *** p < 0.001

Conclusion

Due to the lack of individual data in the report, male hormone values after 4 days of treatment are difficult to evaluate. However, the data are generally in line with the results obtained after a treatment duration of 4 weeks. For females, the tendencies are comparable between the hormone levels measured at 4 days and at 4 weeks. In females cyclicity data serve to support the conclusion that some hormonal imbalances were already present after 4 days of treatment. Qualitatively and quantitatively the effects were stronger in females than in males. The following general conclusions can be drawn.

In both males and females androgen steroids were increased and adrenal steroids (corticosterone and aldosterone) were decreased, while ACTH was increased.

In females estradiol was decreased and LH and FSH were upregulated, especially during dioestrus. Exposure at 1500 ppm was sufficient to reduce estradiol levels to values usually observed during dioestrus in controls. Hormonal changes and thus enzyme inhibition occurred fast enough to explain the rapid abolishment of normal cyclicity in treated females.

In males and in females during prooestrus prolactin was decreased.

The changes observed in androgen, estradiol, LH and FSH levels can be explained by an inhibition of aromatase enzyme activity by the test substance. Aromatase converts both testosterone and androstendione to estradiol. The inhibition of aromatase leads to an increased concentration of androgens and a decreased concentration of estradiol. The decreased estradiol levels trigger a feedback response in the hypothalamic–pituitary axis resulting in increased LH and FSH levels.

The changes concerning adrenal steroids (corticosterone and aldosterone) and ACTH can be explained by a test substance-related decrease of the adrenal enzyme activity of either 11- or 21-

hydroxylase. Reduction in the activity of these enzymes would result in a decrease of corticosterone and aldosterone production, without affecting testosterone synthesis. The decreased adrenal steroid levels trigger a feedback response in the hypothalamic–pituitary axis resulting in increased ACTH levels.

(Draft Assessment Report, Reference 2)

Report:	Wuttke W. 1995;TOX2003-1861 Reg.No. 205 259 (triazole) - *in vitro* investigations into the effects of triazole on the production of ovarian and adrenal steroids and of the pituitary hormone prolactin Georg-August-Universität; Göttingen; Germany Fed.Rep. unpublished BASF DocID 95/11377
Data taken from:	Draft Assessment Report, Reference 2.
GLP:	No, not subject to GLP regulations
Guideline:	There are no guidelines for this study type but the study is considered scientifically valid
Deviations:	Not applicable
Acceptability:	The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole, purity 93.2 %, batch CP 2431/III

Test system: *in vitro*, cultures of different cell types

The effects of epoxiconazole on the production of estradiol, progesterone, corticosterone, cortisol, 17-OH-progesterone and prolactin were investigated *in vitro*. Several cultures of different cell types were used to determine the production of the above mentioned hormones in the absence and presence of the test substance.

Cultures of human granulosa cells were obtained by puncturing human chorionic gonadotropin (HCG)-stimulated human follicles under standard conditions under the *in vitro* fertilisation program. Cultures of rat granulosa cells were obtained from 28 day old Sprague-Dawley rats. Cultures of rat pituitary and adrenocortical cells were obtained from female Sprague-Dawley rats weighing 300 – 350 g. Cultures of porcine luteal and adrenal cells were obtained from adult female pigs.

Estradiol production was determined in rat and human granulosa cells as well as in porcine luteal cell cultures. Rat and human granulosa cells were stimulated by adding 6 ng/mL HCG to the cell medium. Androstenedione (0.01 µmol/L) was used as a substrate for estradiol production by aromatase enzyme activity. The test substance concentrations used ranged from 0.01 – 10 µmol/L.

Progesterone production was determined in porcine luteal cell cultures. The test substance concentrations used ranged from 0.01 – 10 µmol/L.

Progesterone and corticosterone production were measured in rat adrenal cell cultures with and without stimulation by 0.1 µmol/L ACTH. The test substance concentrations used ranged from 0.01

– 1 µmol/L. Ketoconazole, a known inhibitor of adrenal steroid synthesis, was used as a positive control in these assays.

Cortisol and 17-OH-progesterone production were measured in porcine adrenal cell cultures with and without stimulation with 0.1 µmol/L ACTH. The test substance concentrations used ranged from 0.01 – 1 µmol/L. Ketoconazole was used as a positive control.

Prolactin production was determined in rat pituitary cell cultures with and without stimulation by 0.1 µmol/L TRH (thyreotropin-releasing hormone). The test substance concentrations used ranged from 0.01 – 1 µmol/L. The secretion of prolactin was inhibited by dopamine which was used as a positive control.

Hormones were determined by RIA or ELISA methods.

Findings

The results of the different assays were presented in the report by bar graphs as percent of basal secretion (non-stimulated cell cultures, in absence of test substance). Therefore, the figures given in Table 32, 33 and 34 are approximations and not exact figures.

Table 32. Effects on estradiol production

Epoxiconazole concentration (µmol/L)	Estradiol (% basal secretion)		
	Rat granulosa cells	Human granulosa cells	Porcine luteal cells
Basal level	100	100	100
0 (DMSO)	425	360	130
0.01	--	--	75
0.1	125	--	25
1	100	250	25
10	--	--	20

--: not determined

Epoxiconazole is an inhibitor of aromatase activity in cells of all three species investigated. Quantitatively there are differences between rat granulosa cells, porcine luteal cells and human granulosa cells. A concentration of 0.01 µmol/L of epoxiconazole reduced the estradiol production by approximately 50 % in porcine luteal cells. In rat granulosa cells the concentration of 0.01 µmol/L was not tested, but at a concentration of 0.1µmol/L estradiol production was reduced by approximately 70 %. In human granulosa cells a concentration of 1 µmol/L was needed to inhibit estradiol production by 30 %. Thus, the relative sensitivity to the aromatase inhibition by epoxiconazole treatment is: human granulosa cells < rat granulosa cells < porcine luteal cells.

Table 33. Effects on progesterone and 17-OH-progesterone production

Epoxiconazole concentration (µmol/L)	% of basal secretion		
	Progesterone Porcine luteal cells	Progesterone Rat adrenal cells	17-OH-progesterone Porcine adrenal cells
Basal level	--	100	100
0 (DMSO)	100	2000	330
0.01	90	1900	330
0.1	80	1750	370
1	70	2200	450
10	40	--	--
Ketoconazole 1 µmol/L	--	1000	120

--: not determined

There was no effect of epoxiconazole on the progesterone production in rat adrenal cells up to a concentration of 1 µmol/L. In porcine luteal cells progesterone production was clearly reduced at a concentration of 10 µmol/L. The effects of epoxiconazole on 17-OH-progesterone production in porcine adrenal cells are marginal. At 10 µmol/L there appears to be a slight increase, but at the lower concentrations no increase can be seen.

Table 34. Effects on cortisol and prolactin production

Epoxiconazole concentration (µmol/L)	% of basal secretion		
	Cortisol Porcine adrenal cells	Corticosterone Rat adrenal cells	Prolactin Rat pituitary cells
Basal level	100	100	100
0 (DMSO)	500	825	210
0.01	500	825	190
0.1	480	875	220
1	180	925	225
Ketoconazole 1 µmol/L	125	100	--
Dopamine 1 µmol/L	–	–	100

–: not determined

Cortisol production was clearly inhibited at a concentration of 1 µmol/L. At lower concentrations there were no effects. Epoxiconazole had no effect on corticosterone production in rat adrenal cells or on prolactin production in rat pituitary cells.

Conclusion

Epoxiconazole is an inhibitor of aromatase activity in cells of all three species investigated. Quantitatively there are differences between the degree of inhibition in rat granulosa cells, porcine luteal cells and human granulosa cells. The relative sensitivity to the aromatase inhibition by epoxiconazole treatment is: human granulosa cells < rat granulosa cells < porcine luteal cells. However, it is not clear whether this difference exists *in vivo*.

Epoxiconazole has a moderately pronounced inhibitory effect on the 17-hydroxylase enzyme activity. The *in vitro* study found no effect of epoxiconazole on the secretion of prolactin by rat pituitary cells.

(Draft Assessment Report, Reference 2)

Report:	Wuttke W. 2001;TOX2003-1863 Effects of BAS 480 F on the aromatase activity in cultivated human and rat granulosa cells Georg-August-Universität, Göttingen, Germany Fed. Rep. unpublished BASF DocID 2001/1017365
Data taken from:	Draft Assessment Report, Reference 2.
GLP:	No
Guideline:	There are no guidelines for this study type, but the study is considered scientifically valid.
Deviations:	Not applicable
Acceptability:	The study is considered to be acceptable.

Material and Methods

Test material: Epoxiconazole; purity and batch not specified in report.

Test system: *in vitro*, cultures of rat and human granulosa cells

The effects of epoxiconazole on aromatase enzyme activity in rat and human granulosa cells were investigated *in vitro*. Cultures of human granulosa cells were obtained by puncturing human follicles under standard conditions under the *in vitro* fertilisation program. Cultures of rat granulosa cells were obtained from prooestrus Wistar rats. Epoxiconazole was dissolved in ethanol and applied to the cell cultures at concentrations ranging from 10^{-4} to 10^{-8} M.

The release of estradiol, in the presence of 10 nM aromatase substrate 4-androstenedione, from cultivated rat or human granulosa cells was determined directly from the culture medium using immunological methods in a fully automated analysis system and concurrent control sera. A test using the tetrazolium salt MTT was performed to assess possible cytotoxicity of epoxiconazole to the granulosa cells. Vorozole, a known aromatase inhibitor was used as a positive control. The effects of ethanol, the vehicle for the test substance, on aromatase activity were also determined.

Findings

Vorozole (10^{-7} M) as an established aromatase inhibitor led to an inhibition of the aromatase activity in both human and rat granulosa cells (Figure 2a and 2b). On account of the high test

concentrations of epoxiconazole investigated, applications in a solution in ethanol could not be avoided. Therefore, the effect of the pure solvent ethanol was also investigated. A significant decrease in aromatase activity induced by ethanol was seen in rat granulosa cells (see Figure 2b), but not in human cells (see Figure 2a).

Figure 2a. Influence of vorozole on the aromatase activity of human granulosa cells (as in Wuttke, 2001)

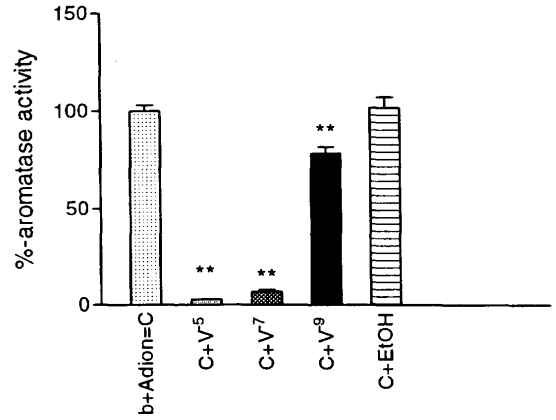


Figure 2b. Influence of vorozole on the aromatase activity of rat granulosa cells (as in Wuttke, 2001)

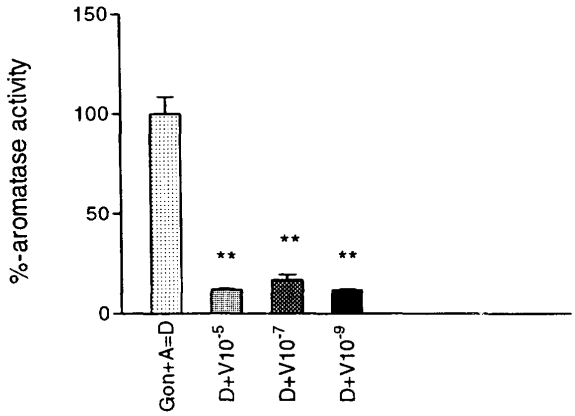


Fig.2a, b. Human and rat granulosa cells were incubated with vorozole (V) for 48 h under standard conditions. For the determination of the aromatase activity in human granulosa cells (fig 2a), 4-androstenedione (10 nM) was added to all experimental preparations. The influence of the solvent, ethanol, was also investigated. The aromatase activity of the untreated control (b+Adion=C) was set at 100% in each case. ** P<0.01 vs. b+Adion

For the determination of the aromatase activity in rat granulosa cells (fig. 2b), gonadotropin (Gon=LH/FSH; 10 mU/ml) and 4-androstenedione (A=10 nM) were added to all test preparations. The aromatase activity of the control (Gon+A=D) was set at 100%. ** P<0.01 vs. Gon+A

Epoxiconazole induced an inhibition of the aromatase activity in human granulosa cells. The concentrations necessary for a significant inhibition were however rather high (> 10^{-6} M); the effect of epoxiconazole was thus less than that of the positive control, vorozole. The question whether the large inhibition of the aromatase activity achievable with 10^{-4} M epoxiconazole leads to considerable cell damage was investigated by means of a normal cytotoxicity test (MTT test). The

result indicates a restriction of cell vitality, which was however not significant on account of the small number of observations (n=3).

In rat granulosa cells, epoxiconazole induced a significant inhibition of the aromatase in all concentrations tested. The rat reacted more sensitively to epoxiconazole than humans and also showed a higher sensitivity to vorozole.

With epoxiconazole, a small reduction in aromatase activity in human granulosa cells was seen at the two lowest concentrations. However, the reduction was only significant at the highest concentration (10^{-4} M; see fig. 3a). The inhibition was then almost as large as the inhibition induced by the positive control. At this concentration, MTT uptake of the cells was reduced by approximately 20 %, indicating beginning cytotoxicity. At the lower concentrations no cytotoxicity was seen.

In rat granulosa cells, significant inhibition of aromatase activity was observed at all tested concentrations (see fig. 3b). The inhibition at all concentrations was almost identical to the one induced by the positive control. The findings may be partly attributed to the inhibitory effect of the solvent ethanol. The concentration range of 10^{-8} M to 10^{-6} M epoxiconazole was not associated with cytotoxicity to rat granulosa cells.

Figure 3a. Influence of epoxiconazole on the aromatase activity of human granulosa cells (as in Wuttke, 2001)

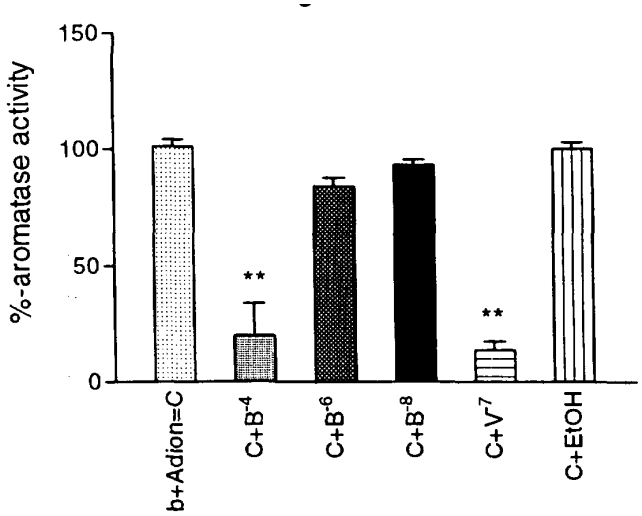


Figure 3b. Influence of epoxiconazole on aromatase activity of rat granulosa cells (as in Wuttke, 2001)

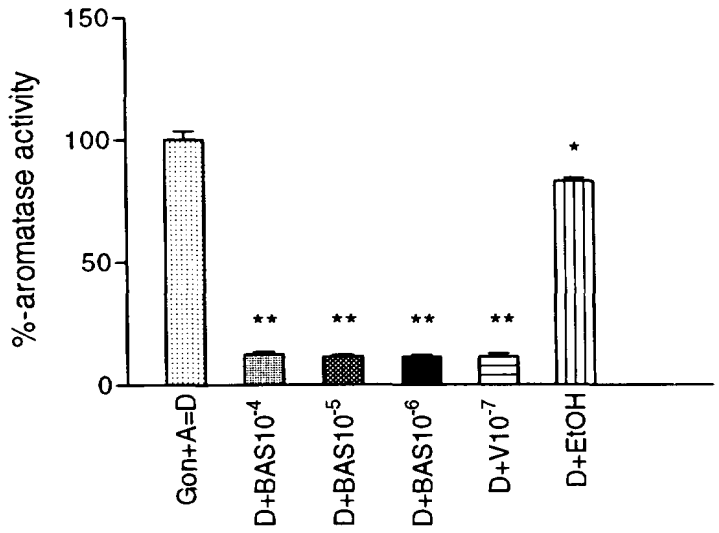


Fig.3a,b. Human granulosa cells (fig 3a) were incubated with epoxiconazole (B) for 48 h under standard conditions. For the determination of the aromatase activity, 4-androstenedione (10 nM) was added to all experimental preparations. The influence of the solvent, ethanol, was also investigated (C+EtOH). As a further control, some of the cultures were treated with vorozole (positive control) in each preparation. The aromatase activity of the untreated control (b+Adion=C) was set at 100% in each case. ** P<0.01 vs. b+Adion

For the determination of the aromatase activity in rat granulosa cells (fig 3b), gonadotropin (Gon=LH/FSH, 10 mU/mi) and 4-androstenedione (A=10 nM) were added to all test preparations. The influence of the solvent, ethanol, was also investigated (D+EtOH). As a positive control, some of the cultures were treated with vorozole (10- M; D+V7). The figure shows the resuits of 3 different preparations, for which triple determinations were carried out in each case (n=3 only applies to D+EtOH). The aromatase activity of the control (Gon+A=D) was set at 100% in each case. ** P<0.01 vs. D

Conclusion

Epoxiconazole is an effective inhibitor of aromatase activity. Differences between human and rat granulosa cells were seen, the inhibitory effect being more pronounced in rat cells (lower concentrations needed). It can be concluded that human granulosa cells are less sensitive than rat granulosa cells for the effect of epoxiconazole on aromatase activity, but it is not clear whether these *in vitro* results reflect the situation *in vivo*.

(Draft Assessment Report, Reference 2)

Report: Birkhøj Kjaerstad M. *et al*. 2007, Pesticides Research No. 111, Danish Environmental Protection Agency. Effects of azole fungicides on the function of sex and thyroid hormones

GLP: Not stated

Guideline: No

Acceptability: Acceptable/Non-guideline

New study not included in the Draft Assessment Report.

Materials and methods

Epoxiconazole and three other azole fungicides were tested using a battery of cell assays for well-known mechanisms of endocrine disruption. Only the results from epoxiconazole are presented here. For further details on materials and methods, see Birkhøj Kjaerstad M. *et al*. 2007.

- Estrogen/anti-estrogen testing using MCF-7 cells

The assay is based on a human breast cancer cell line. The cells depend on estrogen for growth, and proliferation of the cells is an indication of the presence of estrogen like compounds. Epoxiconazole was tested in at least three independent experiments and at 22 different concentrations between 0.001 and 150 μM in triplets.

- Aromatase testing using MCF-7 cells

The MCF-7 cells express the enzyme aromatase naturally. Aromatase converts testosterone to estrogen and hence induce proliferation of the cells. By co-treating the cells with testosterone and the test compound an inhibition or stimulation of the enzyme activity can be registered. Epoxiconazole was tested in three independent experiments at 16 different concentrations between 0.001 and 100 μM in triplets.

- Cytotoxicity in MCF-7 cells

Cytotoxicity of epoxiconazole was evaluated using the Promega Cytotox 96 Non Radioactive Cytotoxicity assay.

- Androgen/anti-androgen testing using the androgen receptor (AR)-assay

Effects on AR activity were tested in a reporter gene assay with minor modifications, using Chinese Hamster Ovary cells (CHO K1). Epoxiconazole was tested at 12 different concentrations between 0.025 and 50 μM, combined with 0.1 nM of the AR-agonist R1881 (NEN, Boston, MA).

- Ah receptor testing using the CALUX assay

Ah receptor testing was performed using rat hepatoma H4IIE cells, stably transfected with the PAH/HAH-inducible luciferase expression vector pGudLuc1.1. The vector contains the firefly luciferase gene under PAH/HAH-inducible control of four murine dioxin responsive elements (DREs) inducing luciferase in a time- and dose-dependent manner. Cells were exposed to epoxiconazole in different concentrations between 0 and 50 μM, and luciferase activity was determined.

- Steroid hormone synthesis testing using H295R cells

The H295R cell line, derived from human adrenocortical carcinoma cells, produces a wide range of steroid hormones in measurable quantities (incl. testosterone, progesterone and estradiol), and can therefore be used a screening assay to detect effects on steroidogenesis. Epoxiconazole was tested in different concentrations between 0 and 30 μM.

- Thyroid testing using GH3 thyroid assay (T-screen)

The assay is based on the thyroid dependent cell growth of a rat pituitary tumour cell line (GH3). The cell line expresses intracellular thyroid receptors (TR) in very high amounts and the assay can be used to study interference of compounds with thyroid hormone at cellular level. Different concentrations, between 0 and 30 μM, of epoxiconazole were tested.

Findings

Epoxiconazole inhibited MCF-7 cell proliferation induced by 10 pM 17β-estradiol, which shows that it has weak anti-estrogenic activity. The lowest observed effect concentration (LOEC) causing a continuously statistically significant response was 25µM. The inhibitory concentrations (IC) were: IC25 = 36 µM; IC50 = 49 µM; IC75 = 66 µM. Cytotoxicity was only detected at the highest concentrations (100, 125 and 150 µM) and hence does not explain the results seen. In addition, epoxiconazole increased the cell proliferation indicating a weak estrogenic activity, and further testing indicated that the proliferation was induced directly via the estrogen receptor (ER).

In the aromatase testing, epoxiconazole inhibited the testosterone induced cell proliferation (LOEC = 1 µM; IC25 = 4 µM; IC50 = 17 µM; IC75 = 73 µM). The concentrations needed to reduce the testosterone induced response were lower than the concentrations needed to reduce 17β-estradiol, indicating that epoxiconazole has both aromatase inhibiting and anti-estrogenic properties, but that the aromatase inhibition dominates at low concentrations.

Epoxiconazole was also shown to have AR antagonistic properties with a LOEC of 0.8µM

The Ah receptor was activated by epoxiconazole with a LOEC of 6.3 µM and a MOEC (maximum observed effect concentration: the lowest concentration showing maximum effect) of 50 µM (9% of max. TCDD, a known AhR agonist, effect).

The production of testosterone and estrogen *in vitro* in H295R cells was inhibited by epoxiconazole, although the inhibiting effect of testosterone was not statistically significant. The inhibiting effect on estradiol production was statistically significant at the three highest concentrations tested (3, 10 and 30 µM).

Conclusion

Epoxiconazole was tested for endocrine disrupting effects *in vitro* using a battery of cell assays. The results show that epoxiconazole has anti-estrogenic and estrogenic activity, anti-androgenic activity as well as inhibiting effects on aromatase and that it can activate the Ah receptor *in vitro*. The anti-estrogenic properties were shown through the inhibition of MCF-7 cell line proliferation, through the inhibition of aromatase, which leads to an inhibition of the conversion from androgen into estrogen, lowering the amount of estrogen, and through an inhibited production of estradiol.

Report: Taxvig C *et al*. 2007, Toxicological Sciences, 100(2), 464-473. Endocrine disrupting activities *in vivo* of the fungicides tebuconazole and epoxiconazole.

GLP: Not stated

Guideline: No

Acceptability: Acceptable/Non-guideline

New study not included in the Draft Assessment Report.

See 5.9.2 Developmental toxicity, p. 40ff

Conclusion

Epoxiconazole had a marked foetotoxic effect and the dams in the highest dose group were in general unable to deliver their pups. Only two out of 7 litters were born normally, and the rest was instead included in the Caesarian section group. In female offspring, AGD was increased, indicating a virilising effect. There were indications of an effect on AGD in males, but the effects were not consistent between foetuses and pups or between the AGD and the anogenital index (i.e. the anogenital distance adjusted for weight differences). The increased gestational length is probably due to the increased levels of progesterone that was seen in the dams, and this increase is probably also involved in the virilising effect seen in the female offspring.

The study shows that epoxiconazole has endocrine disrupting effects *in vivo*, resulting in effects on both dams and offspring, and in particular female foetuses and offspring.

5.9.4.2 Maternal toxicity

Report: Schneider S. *et al*. 2001;TOX2003-1852
BAS 480 F - Maternal toxicity study in Wistar rats - Oral administration (gavage)
BASF AG, Ludwigshafen/Rhein, Germany Fed. Rep.
unpublished
BASF DocID 2001/1014916

Data taken from: Draft Assessment Report, Reference 2.

GLP: No
This study, as currently reported, is not in full compliance with GLP principles, and therefore does not have a GLP status.

Guideline: EEC 87/302 B, OECD 414

Deviations: Group size; litter data and foetal parameters not investigated.

Acceptability: The study is considered to be acceptable for characterisation of maternal toxicity.

Material and Methods

Test material: Epoxiconazole, purity 94.7 %, batch 00-2046

Test animals: groups of 10 female Wistar CrlGlxBrlHan:WI rats, provided by Charles River, Sulzfeld, Germany

The aim of this study was to investigate maternal toxicity in pregnant rats in more detail. For this purpose 10 mated female Wistar rats were treated from day 6 through day 19 post coitum (p.c.) with epoxiconazole doses of 0 (control treated with the vehicle double distilled water with 0.5 % carboxymethyl cellulose), 45; 60 and 75 mg/kg bw by gavage at a constant dosing volume of 10 mL/kg bw.

Food consumption and body weights were recorded at regular intervals throughout the study period. The animals' state of health was checked daily. On day 20 p.c., all females were sacrificed and

haematological and clinical chemical parameters were determined. The dams were assessed by gross pathology including determination of liver, kidney and the unopened uterus weights. The foetuses were removed from the uterus and discarded without further examinations.

Findings

Clinical parameters

Vaginal haemorrhages shortly before terminal sacrifice were observed in 4 dams at 75 mg/kg bw and 1 dam at 60 mg/kg bw, while no findings were noted at 45 mg/kg bw.

At the high dose level mean food consumption was reduced (see Table 35), with a concurrent decrease in body weight (days 6 - 20 p.c.), being 11 % below the control animals at study termination. Body weight gain was significantly impaired, especially during the initial treatment phase from days 6 - 8 p.c. and corrected body weight gain was reduced (34 % below control) as was carcass weight (11 % below control).

At the mid-dose level a reduction in mean food consumption was seen together with marginally reduced body weights (n.s.) to 5 % below the control value at study termination. Body weight gain was significantly impaired on study days 19 - 20. Corrected body weight gain was reduced (32 % below control) as was carcass weight (8 % below control).

At the low dose level mean food consumption was reduced (10 % over the entire treatment period). Body weights were marginally lower than control values (6 %) without statistical significance and corrected body weight gain was 17 % below control.

Table 35. Clinical data – maternal toxicity (gavage) study in Wistar rats

Parameter	Dose level (mg/kg bw/d)			
	0	45	60	75
Mated females on study	10	10	10	10
Pregnant females on study	10	9	9	10
Mortality of dams	0	0	0	0
Clinical symptoms	-	-	1 dam: vaginal haemorrhages	4 dams: vaginal haemorrhages
Food consumption day 6-19 (g)	21.3	19.2 Decreased during days 6–8, 13-17**, and 17-20*	19.3 Decreased during days 6-8**, 13-17*, and 17-20**	18.7 Decreased during days 6–8, 13-15*, and 15-20**
Body weight day 20 (g)	291.0	272.5	275.4	260.0**
Body weight gain days 0-20 (g)	126.7	111.9	112.6 Decreased during days 19-20*	105.1* Decreased during days 6-8 **
Corrected body weight gain (g)	39.2	32.7	26.5*	25.8*

* $p < 0.05$; ** $p < 0.01$

Clinical chemistry/haematology

With the exception of platelet counts, which were unaffected at the low dose level, red blood cells, haemoglobin, haematocrit and platelets were significantly decreased in all epoxiconazole-treated groups. Total protein, albumin and globulins were reduced at all dose levels whereas alanine aminotransferase was only diminished at 60 mg/kg bw/d and higher. Aspartate amino transferase and glucose levels were increased at all dose levels.

Organ weights

No effects on kidney weights were seen at any dose level, and the only effect on organ weights seen was an increase in relative liver weight (+8% compared to controls) at the highest dose level.

Table 36. Clinical chemistry/haematology/organ weights - maternal toxicity gavage study in Wistar rats

Parameter	Dose level (mg/kg bw/d)			
	0	45	60	75
Haematology				
Red blood cells (%)	100	91**	85**	81*
Haemoglobin (%)	100	93**	85*	81**
Haematocrit (%)	100	93**	87*	82**
Platelets (%)	100	86	76**	68**
Clinical chemistry				
Alanine aminotransferase (%)	100	94	84*	83**
Aspartate aminotransferase (%)	100	131*	134*	112*
Glucose (%)	100	107*	106*	111*
Total protein (%)	100	88**	85**	83**
Albumin (%)	100	94**	91*	90
Globulin (%)	100	81**	79**	75**
Organ weights				
Liver, absolute (g)	11.63	11.38	11.35	11.25
Liver, relative (g)	3.988	4.179	4.127	4.323**
Kidney, absolute (g)	1.42	1.31	1.32	1.34
Kidney, relative (g)	0.489	0.482	0.482	0.515

* $p < 0.05$; **$p < 0.01$

Conclusion

Decreases in food consumption and body weights were seen after epoxiconazole treatment, with effects being more pronounced in the highest dose group. No foetuses were examined in this study. The low dose used in this study corresponds to the previously determined LOAEL for embryofoetal and maternal toxicity. Haematology results indicated an anaemic effect, and this could possibly lead to embryofoetal toxicity due to reduced oxygen. The reduced number of platelets at the mid and

high dose level is also attributable to the test substance administration. An impairment of liver function can be deduced from clinical chemistry changes, most of which were seen at all dose levels. However, increased liver weight was seen at high dose only.

(Draft Assessment Report, Reference 2)

5.9.5 Summary and discussion of reproductive toxicity

Table 37. Epoxiconazole - summary table of reproductive and developmental toxicity

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
RAT - ORAL EXPOSURE		
Hellwig J. Hildebrand B. 1989, TOX2003-1848, Reg. No. 1989/0477	Prenatal toxicity, gavage (range-finding), Chbb:THOM (SPF) Wistar rats, 0, 20, 60, 180 mg/kg bw/d on days 6-15 post coitum (p.c.), acceptable	<u>Dams</u> 180 mg/kg bw/d: Initial body weight loss, corrected net body weight gain decreased, piloerection (8 dams), reddish nasal discharge (2 dams), fur smeared with urine (2 dams) 60 mg/kg bw/d and above: Body weights (treatment period and first days post treatment) and body weight gain decreased 20 mg/kg bw/d and above: Corrected (net) body weight gain decreased 20 mg/kg bw/d and higher: placental weights increased <u>Foetuses</u> 180 mg/kg bw/d: 3 complete litter losses, cleft palates in 136 out of 271 foetuses (50%) in 18 out of 20 litters (90%), post-implantation loss marginally increased (n.s.) 60 mg/kg bw/d: Post-implantation loss marginally increased (n.s.)
Hellwig J., Hildebrand B.; 1990b, TOX2003-1849, Reg. No. 1990/0214	Prenatal toxicity, gavage, Chbb:THOM (SPF) Wistar rats: 0, 5, 15, 45 mg/kg bw on days 6 – 15 p.c., acceptable	<u>Dams</u> 45 mg/kg bw/d: Food consumption decreased during treatment, body weight gain decrease initially (days 6-8). 15 mg/kg bw/d and above: Placental weights increased <u>Foetuses</u> 45 mg/kg bw/d: Number of resorptions (especially late ones) slightly increased, post-implantation loss marginally increased, number of foetuses with skeletal variations increased (especially rudimentary cervical and/or accessory 14th ribs)
Hellwig J. Hildebrand B. 1992, TOX2003-1847, Reg. No. 1992/10689	Two-generation oral feeding, Chbb:THOM (SPF) Wistar rats, 0, 10, 25, 250 ppm, acceptable	<u>Parents, 250 ppm</u>: Food consumption decreased in F0 females (and during lactation in F1a), F1 males (early pre-mating period) and F1 females (pregnancy/lactation) Body weight decreased in F1 males Adrenal weights decreased in F0 and F1 males Precoital interval increased in three F0 and four F1 mating pairs Vaginal haemorrhage in six F0 dams (pregnancy F1a, two of these died with dystocia), one F1 dam (prolonged pregnancy, no live litter) Duration of pregnancy increased (F0 to F1a, F1 to F2)

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
		Liver weights increased in F1 females without histopathological correlate; liver fatty change decreased in F1 males Offspring, 250 ppm: Number of stillborn pups increased (F1, F2), number of live-born pups decreased (F1), mortality during rearing period increased (F2), viability index decreased (F1b, F2), lactation index decreased (F2), anasarca: F2: 3 pups from 3 litters body wt gain and body wt decreased
Schneider S. *et al*. 2002, TOX2002-2288, Reg. No. 2002/1012810	Prenatal toxicity, comparison of Batch No. 1: 94.1 % Batch No. 2: 99.8 % gavage, CrlGlxBrlHan:WI Wistar rats, 0, 180 mg/kg bw on days 6 - 19 p.c., acceptable	Dams Batch #1: Food consumption decreased (14 %), body weight, body weight gain decreased (19 %), corrected net body weight gain decreased (44 %) Blood in bedding / vaginal haemorrhages (6 dams), piloerection (3 dams). Pregnant uterus weight decreased (14 %). Batch #2: Food consumption decreased (6 %), body weight gain decreased (4 %) and corrected body weight gain decreased (30 %) Blood in bedding / vaginal haemorrhages (2 dams), piloerection (1 dam). Batch #1 and Batch #2: Red blood cells, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, number of platelets decreased, clotting time increased ALT, AP decreased; AST increased, protein content decreased (total protein, albumin, globulins), serum K, Mg decreased, inorganic phosphate, urea increased (several parameters more pronounced for batch no 1) Estradiol, progesterone and prolactin decreased (no difference between batches). Based on group means, effects of batch no 1 were more pronounced. Based on number of affected dams, both batches showed similar toxicity. Foetuses Batch no 1 and batch no 2: Placental weights increased, resorption rate (especially late resorptions) increased, post-implantation loss 40 - 60 % in test groups versus 10 % in control, number of live foetuses decreased Batch no 1: incidence of absent or small tuberositas deltoidea increased.
Taxvig *et al.* 2007	*In vivo* investigation of endocrine disrupting activities, acceptable	Dams Gestational length increased, dystocia, levels of progesterone and testosterone increased

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
		Foetuses/pups Increased loss of foetuses and postnatal death of pups Incidence of stillbirths increased, live litter size decreased Increased anogenital distance in female foetuses and offspring
RAT - DERMAL APPLICATION		
Hellwig J., Hildebrand B. 1993, TOX2003-1850, Reg. No. 93/10151	Prenatal dermal toxicity, Chbb:THOM (SPF) Wistar rats 0, 100, 400, 1000 mg/kg bw/d on days 6 - 15 p.c., acceptable	Dams No signs of toxicity Foetuses 1000 mg/kg: Placental weights increased, cleft palate: 1 foetus, skeletal variations increased (rudimentary cervical and/or accessory 14th rib(s)
RABBIT - ORAL EXPOSURE		
Hellwig J., Hildebrand B. 1990a, TOX2003-1851, Reg. No. 90/0213	Prenatal toxicity, gavage, Chbb:HM Himalayan rabbits, 0, 5, 20, 80 mg/kg bw/d on days 7 - 19 post-insemination, acceptable	Dams 80 mg/kg bw/d: Pregnant uterus weights slightly decreased, total litter loss three does 20 mg/kg bw/d and above: food consumption slightly decreased during treatment period, body weight gain decreased, abortion in one doe Foetuses 80 mg/kg bw/d: Placental weights slightly decreased (8 %). Post-implantation loss/ resorption rate increased including the three does without viable foetuses.
ENDOCRINE DISRUPTIVE PROPERTIES		
Mellert W. 1992, TOX2003-1857, Reg. No. 1992/10715 (Interim report) and Mellert W., Hildebrand B. 1999, TOX2003-1858, Reg. No. 1999/11334 (Amendment)	Determination of hormone concentrations after 4 - 6 days and 28 days exposure, Chbb:THOM (SPF) Wistar rats, 0, 1500, and 3000 ppm, 200mg/kg bw/d, acceptable	200 mg/kg bw/d Males and females: body wt loss Females: reduced general state of health, prolonged oestrous cycles 3000 ppm Males: decreased corticosterone and prolcatin levels Females: decreased corticosterone levels during prooestrus 1500 ppm and above Males and females: reduced food consumption Males: increased androgen and FSH levels Females: increased androgen levels, prolonged oestrous cycles, decreased estradiol levels, decreased aldosterone and prolactin levels during prooestrus
Wuttke W. 1995, TOX2003-1861, Reg. No. 1995/11377	*In vitro* investigations of effects on ovarian, adrenal and pituitary hormones, different cell types (rat, pig,	Aromatase inhibition Porcine luteal cells > rat granulosa cells > human granulosa cells Progesterone production

Author(s)/Year, RMS Report ID, Company Report ID	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
	human) 0.01 – 10 µmol/L, acceptable	Inhibited in porcine luteal cells, not in rat adrenal cells Corticoid production Inhibited in porcine adrenal cells, not in rat adrenal cells Prolactin production No inhibition in rat pituitary cells
Wuttke W. 2001, TOX2003-1863, Reg. No. 2001/1017365	*In vitro* investigations of effects on aromatase activity, granulosa cells (rat, human) 0.01 – 100 µmol/L, acceptable	Aromatase inhibition Rat granulosa cells: at 10^{-7} M and higher Human granulosa cells: at 10^{-4} M
Birkhøj Kjaerstad *et al*. 2007	*In vitro* investigations of effects on the function of sex and thyroid hormones, acceptable	Weak anti-estrogenic/estrogenic activity In human breast cancer cells (MCF-7 cells) Aromatase inhibition and anti-estrogenic properties In human breast cancer cells (MCF-7 cells), aromatase inhibition dominates at low concentrations Androgen receptor (AR) antagonist In gene assay using Chinese Hamster Ovary cells Ah receptor activator In rat hepatoma cells Inhibtion of estradiol production Using the H295R cell line, derived from human adrenocortical carcinoma cells, also inhibition of testosterone production, but not significant
MATERNAL TOXICITY		
Schneider S. *et al*. 2001, TOX2003-1852, Reg. No. 2001/1014916	Maternal toxicity, gavage, CrlGlxBrlHan:WI Wistar rats, 0, 45, 60, 75 mg/kg bw on days 6 - 19 p.c., acceptable	75 mg/kg bw/d Liver weights increased 60 mg/kg bw/d and higher Body weight gain decreased days 19 - 20, corrected net body weight gain decreased Vaginal haemorrhage (4 dams at 75 mg/kg and one dam at 60 mg/kg) Platelets and alanine aminotransferase decreased 45 mg/kg bw/d and above: Food consumption decreased Red blood cells, haemoglobin and haematocrit decreased, total protein, albumin and globulins decreased, aspartate amino transferase and glucose levels increased

Data on reproductive and developmental toxicity and endocrine disruption studies are summarised in Table 37.

The reproductive and developmental toxicity of epoxiconazole was investigated in a two-generation reproduction study (rat), a prenatal toxicity study (rat), preceded by a range-finding study, two maternal toxicity studies (rat; one without evaluation of the foetuses), a prenatal development toxicity study (rabbit), a dermal prenatal toxicity study (rat) and an additional developmental toxicity study (rat), with special focus on endocrine disrupting effects. In addition, several studies on the endocrine disrupting effects *in vivo* and *in vitro* were performed.

In several reproductive toxicity studies with oral exposure in rats, an increase in post-implantation loss, resorption rate (in particular late resorptions), number of stillborn pups, and postnatal deaths was seen, with a concurrent decrease in number of liveborn foetuses/live litter size and in viability index. Embryofoetal toxicity was seen as a decrease in viability index in F2 pups from a dose of 10 ppm. Increased embryo- or foetolethality was also apparent at 45 mg/kg and surviving foetuses at that dose were found to have a higher rate of skeletal variations (additional cervical and thoraco-lumbar rib elements). At 180 mg/kg bw/d increased post-implantation loss was observed consistently in two studies with different Wistar rat strains. This effect was more pronounced when treatment was extended to cover the foetal as well as the post-implantation embryonic period and thus may have affected the ability to detect malformations.

In the range-finding study (Hellwig & Hildebrand, 1989) most foetuses survived exposure during the embryonic period and were found to have a very high incidence of cleft palates at term, affecting 90% of litters and 50% of pups. This finding also existed, but at a much lower incidence, in the second high-dose study which compared two different epoxiconazole batches (Schneider *et al*, 2002) In this study, embryo/foetolethality reached about 50% and it must be assumed that conceptuses with oral clefts were preferentially eliminated by death and resorption during the foetal period. Cleft palate was seen in one pup after dermal exposure of 1000 mg/kg bw/day in rats (Hellwig & Hildebrand, 1993). Some surviving foetuses in the study where the two different batches were compared had anasarca, generalised oedema, with or without domed head. The more impure of the two batches also induced absence or reduction of the tuberositas deltoidea, a part of the humerus. Anasarca was also seen in three pups in three different litters in the two-generation study (Hellwig & Hildebrand, 1992). In rabbits (Hellwig & Hildebrand, 1990a) no malformations were seen, however, 3 of 13 does at 80 mg/kg bw/d had resorbed all implants and 3 further does had more than two dead implants, and hence the post-implantation loss was markedly increased for this group. These effects are evidence of developmental toxicity, but can also mask teratogenic effects such as malformations.

In the developmental toxicity focused on endocrine disrupting effects (Taxvig *et al*, 2007) anogenital distance was increased in both female foetuses at GD 21 and in newborn female offspring. There were indications of an effect on AGD in males, but the effects were not consistent between foetuses and pups, or between the AGD and the anogenital index (i.e. the anogenital distance adjusted for weight differences). No effects of epoxiconazole on the measured hormone levels in foetuses on GD 21 were found. In plasma from treated dams, an increase in progesterone level (7-fold) and an increase in testosterone (2-fold) were seen. A tendency towards lowered estradiol levels in female pups on PND 16 was seen, but this was not statistically significant. The increased levels of progesterone that was seen in the dams are probably involved in the increased gestational length, and probably also in the virilising effect seen in the female offspring. There was also a tendency (n.s.) towards a decrease in testosterone in males at PND 16.

In dams signs of toxicity were observed in the dose range between 20 and 180 mg/kg/d. At 15 mg/kg bw/d, no maternal toxicity was noted. At 20 mg/kg bw/d effects were slight and the signs of

maternal toxicity consisted of reduced food consumption and lower corrected body weight gain. Effects became more severe with increasing doses resulting in reduced food consumption, body weight loss and piloerection. When the scope of test parameters for assessment of maternal toxicity was expanded to include haematological, clinico-chemical and organ weight determinations, indications of anaemic effects and changes in liver enzyme levels was observed in the dose range between 45 and 180 mg/kg bw/d. Liver weight was affected only at the highest dose level in this study. A study conducted only at the high dose level of 180 mg/kg bw/d also demonstrated an influence on hormone serum levels of the dams and showed reductions for estradiol, progesterone and prolactin. In several studies examining effects on hormone levels, increases in androgens, FSH, LH and ACTH and decreases in estradiol and corticosterone have been seen, and this is in part thought to be attributed to the aromatase inhibiting effects of epoxiconazole.

Placental weights were increased at doses of 15 mg/kg bw/d and above. At the LOEL this increase was slight (8 %), but at 180 mg/kg bw/d increases of more than 125 % were observed. Possible reasons for this finding have not been elucidated. The increase in placental weight may be related to the hormonal changes induced in the dams and indicate increased placental metabolic function (synthesis of steroids, detoxification of epoxiconazole). In the absence of histopathological data, the increase in placental weight is not considered adverse as long as there are no adverse effects on foetal parameters (foetal weight, foetal morphology, viability).

The toxicokinetic data showed that for both sexes, C_{max} increased slightly less than proportionally with dose, whereas the AUC was linear over the tested dose range, indicating that the absorption process was not saturated at the high dose level. Epoxiconazole was widely distributed in the organism, with highest residues found in blood, liver, kidneys, spleen, lung, and adrenals. Overall, at 168 h post-dosing, only small amounts of radioactivity were detected in these organs, while only whole blood (but not plasma) and spleen levels were declining more slowly, indicating some kind of binding to blood cells by either the parent substance or its metabolites (some effort was undertaken to prove the latter, but interpretation of results was seen as questionable).

Conclusion

The reproductive effects of epoxiconazole have been evaluated in a number of studies, and a marked increase in post-implantation loss, number of stillborn pups and number of resorptions have been seen in several of these. A very high incidence of cleft palates was seen in one of the studies, while lower incidences of this malformation were seen in others. Increased post-implantation loss is a sign of developmental toxicity, but can also mask teratogenic effects such as malformations.

A majority of the effects in offspring were seen at dose levels where signs of toxicity were seen in dams, and it is known that maternal toxicity can cause adverse effects in offspring. However, several studies have investigated the connection between maternal and offspring toxicity, and studies where maternal stress has been induced have been performed in order to evaluate the effect of maternal toxicity on foetuses/offspring. It has been concluded that effects on foetuses can not be automatically discarded even at dose levels where maternal toxicity is seen, but a thorough evaluation of the effects has to be done on a case-by-case basis. In Fleeman *et al* (2005; Reference 7) the effects of severe feed restriction in dams, leading to substantial weight loss, on embryo-foetal development was evaluated and no increase in external, visceral or skeletal malformations was seen, but only an increase in skeletal variations. The relationship between parental and developmental toxicity has also been discussed by the Specialized Experts (ECBI/30/04; Reference 8) and they concluded that: “Severe malformations in the foetus even at marked maternal toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) should not be dismissed for classification. Some other observations in the offspring (e.g. embryonic death, reduced body weight, delays in ossification) seen only at dose levels causing marked maternal toxicity are not

relevant for classification purposes. However these dose levels could mask a potential to cause malformations, which could be of concern without full information on the dose response relationship for maternal and developmental toxicity." For further information, see Reference 8.

When comparing the effects after epoxiconazole exposure with the effects seen with several other triazole fungicides used today, there is a consistency between them. Malformations, and especially cleft palates, which were also seen with epoxiconazole, is a common effect in this group of compounds, and the background incidence of this malformations in rats is low.

Endocrine disruptive effects through different mechanisms have been seen both *in vitro* and *in vivo*, and effects are seen at relatively low doses. In Taxvig *et al*, 2007, endocrine disruption *in vivo* was studied, and effects were seen on e.g. the anogenital distance, a sensitive end-point for evaluation of endocrine disrupting effects, which has not been studied previously.

After evaluating the effects seen after epoxiconazole exposure in dams and offspring, our conclusion is that the effects seen on developmental end-points support a classification of epoxiconazole as a **Repr Cat 2; R61 "May cause harm to the unborn child" based on Directive 67/548/EEC criteria.**

(Note: No change to the current classification as Repr Cat 3; R62 "Possible risk of impaired fertility" is proposed.)

5.10 Other effects

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not relevant for this dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Epoxiconazole is a widely used fungicide, and human exposure occurs through occupational use and through diet for consumers. The substance has been discussed in the Technical Committee (TC C&L) under Dir. 67/548/EEC and was included in the 28th ATP as Repro Cat. 2; R61. This classification was revised in the 29th ATP and epoxiconazole is currently classified as Repr. Cat 3; R63 in Annex I to Dir. 67/548/EEC.

In May 2007, a possible re-opening of the discussion on epoxiconazole was considered under "General issues" at a meeting of the TC C&L. Four countries then voted for a re-opening, and ECB concluded that "In case a member state considers that, on the basis of new data on endocrine disruption, there is a need to re-classify epoxiconazole, they should send a proposal in Annex XV format to ECHA" (Follow-up V of the meeting of the Technical committee on classification and labelling in Arona, 15-16 May 2007, Reference 5). New studies have become available since the Draft Assessment Report was finalised and since the present classification was agreed on, where effects on developmental end-points not previously evaluated for epoxiconazole are seen. Endocrine disrupting chemicals (EDCs) are a growing concern world-wide, and the new data shows that epoxiconazole is a potent endocrine disruptor, e.g. as an inhibitor of aromatase with concomitant effects on developmental end-points. The endocrine disruptive potential of epoxiconazole has been shown in several studies both *in vivo* and *in vitro*, including studies with human cell lines indicating its relevance to humans.

It should be noted that these new studies have **not** been peer-reviewed by EFSA (see the recently finalised "Conclusion regarding the peer review of the pesticide risk assessment of the active substance epoxiconazole", finalised 26 March 2008, reference 6), i.e. EFSA has not conducted their own evaluation of the studies, but instead followed the classification included in Annex I of Dir. 67/548/EEC.

There are many triazole fungicides used today, of which some are considered reproductive toxicants (with various potency), while some have not shown any effects at all on reproduction. Developmental effects seen with several of the reproductively toxic compounds in this group are malformations, and especially cleft palates, which was also seen with epoxiconazole.

It is proposed that epoxiconazole is classified as Repr. Cat. 2; R61, according to Directive 67/548/EEC criteria. Harmonised classification and labelling of reprotoxicants is considered a Community-wide action under Article 115 in REACH and it is recommended that the classification proposal is considered for inclusion in Annex I of Directive 67/548/EEC.

OTHER INFORMATION

For all references, see REFERENCES.

REFERENCES

Reference 1. List of end-points, Epoxiconazole, EPCO Manual E4-rev 4 (September 2005), European Food Safety Authority (EFSA). (Attached to the dossier.)

Reference 2. Draft Assessment Report, Annex B, B-6: Toxicology and metabolism; 18 april 2005, Epoxiconazole, Rapporteur Member State: Germany. (Attached to the dossier)

Reference 3. Taxvig, C., Hass, U., Axelstad, M., Dalgaard, M., Boberg, J., Raun Andeasen, H. and Vinggaard, AM. 2007 Endocrine-disrupting activities *in vivo* of the fungicides tebuconazole and epoxiconazole, Toxicological Sciences 100(2), 464-473. (Attached to the dossier.)

Reference 4. Birkhøj Kjaerstad, M., Raun Andeasen, H., Taxvig, C., Hass, U., Axelstad, M., Metzdorff, S. and Vinggaard, AM. 2007 Effects of azole fungicides on the function of sex and thyroid hormones. Pesticides Research No 111, Danish Environmental Protection Agency. (Attached to the dossier.)

Reference 5. Follow-up V of the meeting of the Technical committee on classification and labelling in Arona, 15-16 May 2007. (Attached to the dossier.)

Reference 6. EFSA Scientific Report (2008) 138, Conclusion of the peer review of the risk assessment of the active substance epoxiconazole. Finalised: 26 March 2008 (version without end-points), European Food Safety Authority (EFSA). (Attached to the dossier.)

Reference 7. Fleeman, TL., Cappon, GD., Chapin, RE and Hurtt, ME. 2005 Effects of feed restriction during organogenesis on embryo-foetal development in the rat. Birth Defects Research (Part B) 74:442-449. (Attached to the dossier.)

Reference 8. Expert discussion on the classification of substances toxic to reproduction, Ispra 4-5 March 2004, ECBI/30/04. (Attached to the dossier.)