European Chemicals Agency

COMMITTEE FOR RISK ASSESSMENT

BACKGROUND DOCUMENT TO THE OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING OF

EPOXICONAZOLE
EC number: 406-850-2
CAS number: 133855-98-8

Final
17 March 2010

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)

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EC Number: 406-850-2

CAS number: 133855-98-8

Summary of the opinion:

Considering all the available data, two main adverse effects of epoxiconazole on development were identified and considered as critical for the classification decision:

- Post implantation loss and resorptions
- Malformations as cleft palates

Post-implantation loss

Several prenatal developmental toxicity studies are available and provide information on the induction of post-implantation loss.

By oral route, whereas no significant increase in post-implantation loss was observed in studies in which rats were exposed to 45 mg/kg/d epoxiconazole (Hellwig 1990b) and to 180 mg/kg/d (Hellwig 1989) from gestation days (GD) 6 to 15, a large increase of post-implantation loss was observed in Schneider 2002 at the same dose of 180 mg/kg/d with an exposure partially extended to the end of gestation (GD 6-19). Resorptions were mainly identified as late resorptions.
In Taxvig 2007 and 2008, in which exposure was entirely extended to the end of gestation (GD7-21), a significant increase in post-implantation loss was observed at 50 mg/kg/d and consisted in late and very late resorptions.
No effect is observed in rat by the dermal route up to 1000 mg/kg/d (Hellwig 1993).
An increase in post-implantation loss was also observed at the highest dose by the oral route in rabbits in presence of maternal toxicity (Hellwig 1990a) and consisted mainly of early loss in contrast to rats.

In the two-generation study (Hellwig 1992) a significant decrease in mean litter size is seen at the highest dose in F1a and F1b that may be consistent with an effect on post-implantation loss.

Altogether, these data indicate that the induction of post-implantation loss by epoxiconazole is worsened with the extension of the duration of exposure at the end of gestation with higher rate of resorptions and later stages of resorptions observed. Post-implantation loss was observed in prenatal developmental toxicity studies, in which dams were sacrificed before parturition. It is considered that dystocia may not have contributed to the induction of resorptions. Induction of post-implantation loss was observed in the Taxvig studies in absence of significant maternal toxicity. Therefore, it cannot be considered secondary to non specific maternal toxic effects. In these studies, maternal toxicity was assessed by measurement of maternal body weight gain and clinical signs but it should be noted that maternal food consumption was not measured.

The hypothesis that this effect could be secondary to endocrine disruptive effects in the mother has been raised. However, no correlation between the progesterone level in dam plasma and the rate of very late resorptions was identified from an analysis of individual data from the Taxvig 2007 and Taxvig 2008 studies. It should however be noted that available data on hormonal effects of epoxiconazole in dams show a consistent significant effect on oestradiol and testosterone levels but not on progesterone. In Schneider 2002 both oestradiol reductions and induction of late resorptions were observed. Besides, another aromatase inhibitor – letrozole - has effects on maternal levels of oestradiol but not on progesterone in monkeys (Albrecht 2000). In rats, letrozole also induces an increase in late resorptions that is prevented by co-exposure to oestrogen (Tiboni 2009). This tends to demonstrate that late resorptions in rats may be linked to endocrine disruptive effect of aromatase inhibitors in the dams via oestradiol. . It can be argued that due to differences in hormonal regulation of gestation between species, a doubt on human relevance could be raised for such a mechanism of action. However, in absence of clear data to establish the mechanism of action of epoxiconazole for induction of late resorptions, **no conclusion can be made on the potential absence of relevance for humans**.

RAC therefore considers that the level of evidence for induction of post-implantation loss is in agreement with the criteria for CLP classification Repr. Cat. 1B that "available data provide **clear** evidence of an adverse effect [..] on development in the absence of other toxic effects or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**". Besides, in the absence of relevant mechanistic information **it cannot be concluded "that there is a doubt about the relevance of the effect for humans**" implying that "classification in category 2 may be more appropriate".

The induction of post-implantation loss by epoxiconazole therefore justifies a developmental classification in Cat. 1B (CLP).

Cleft palates

Several prenatal developmental toxicity studies are available and provide information on the induction of cleft palates.
A very high rate of cleft palates (50% of foetuses, 90% of litters affected) was observed in the rat by oral route in Hellwig 1989 at the high dose of 180 mg/kg/d. Such an increase was not reproduced at the same high dose in Schneider 2002 in none of the two purity batch, with cleft palates observed in only 2 (2.4%) and 1 (0.8%) foetuses. However, in this study, the high rate of post-implantation loss (respectively 59 and 43%) may have masked teratogenic effects. Maternal toxicity was noted at this dose level in both studies as evidenced by decreases in food consumption and significant decrease in corrected maternal body weight gain (-45 and -30%). One cleft palate was also observed at the low dose (20 mg/kg/d) in Hellwig 1989.

In the other prenatal developmental toxicity studies, one cleft palate was also identified at the mid-dose (15 mg/kg/d) in rat by oral route in Hellwig 1990b, one at the high dose (1000 mg/kg/d) in rat by dermal route (Hellwig 1993). Besides, one cleft palate was reported in the two-generation study (Hellwig 1992) at the highest dose in F1b (approx. 23 mg/kg/d). No maternal toxicity was observed at these dose levels in these rat studies.
In the rabbit, one cleft palate was observed at the low dose (5 mg/kg/d) by oral route (Hellwig, 1990a). However, in the absence of such findings at the mid- and high-doses, its significance is unclear.

Cleft palate is a rare malformation with available historical control data in rats showing that 1 foetus with a cleft palate may be spontaneously observed on rare occasions (historical control mean: 0.06%; range: 0-0.2.% in Hellwig 1990b indicating twice 1 cleft palate observed in 10 studies). Occurrence of one cleft palate in one study is therefore consistent with historical controls and cannot be unequivocally attributed to treatment. However, the repetition of this isolated finding in all five rat prenatal developmental toxicity studies that investigate malformations supports the conclusion that they are not of spontaneous origin and that they are biologically significant.

The absence of a dose-response in two of the studies (Hellwig 1989 and Hellwig 1990b) also raises an uncertainty on the relation of this malformation with treatment. However, considering the general low occurrence of this finding, a very large number of animals would be necessary to expect a clear dose-response and the biological significance should be given greater importance.
Besides, cleft palate is a malformation that is commonly observed with triazoles compounds in the presence or in the absence of maternal toxicity. It is a very specific malformation implying a disturbance in the process of craniofacial morphogenesis and several modes of action have been proposed. Menegola 2006 suggest that triazoles may inhibit the embryonic CYP450 (CYP26) involved in the regulation of retinoic acid whereas an alternative hypothesis involving blockade of IKr potassium channel, embryonic arrhythmia and hypoxia has also been proposed, based on data for ketoconazole (Ridley 2006, Danielsson 2007). However, none of these modes of action have been studied for epoxiconazole.

Overall, RAC considers that based on a weight of evidence approach and considering the specificity and the spontaneous infrequency of this malformation otherwise commonly seen with triazoles, the induction of a high incidence of cleft palates in the presence of maternal toxicity (Hellwig 1989) and the repeated observation of isolated cleft palates in rats at doses without maternal toxicity enable **a clear identification of cleft palate as a developmental effect** of epoxiconazole. It is considered that induction of cleft palates cannot be attributed to maternal toxicity such as decreased food consumption or reduced body weight gain and **it cannot be considered secondary to other maternal toxic effects**.

RAC therefore considers that the level of evidence for induction of cleft palates is in agreement with the criteria for CLP classification Repr. Cat. 1B that "available data provide **clear** evidence of an adverse effect [..] on development in the absence of other toxic effects or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**". Besides, in the absence of relevant mechanistic information **it cannot be concluded "that there is a doubt about the relevance of the effect for humans**" implying that "classification in category 2 may be more appropriate".

The induction of cleft palates by epoxiconazole therefore justifies a developmental classification in Cat. 1B (CLP).

Overall conclusion

Based on all the available data and the weight of evidence on the impact of epoxiconazole on developmental toxicity, RAC considers that epoxiconazole has to be classified as Reprotoxic Category 1B (CLP) and Reprotoxic Category 2 (Directive 67/548).

Additional information

During the discussion on epoxiconazole at RAC, BASF announced that they would provide several studies further investigating reproductive toxicity and endocrine disruption for human health assessment. These studies with their final report completion dates are as follows:

- Modified rat prenatal developmental toxicity study with epoxiconazole with GD18 and GD21 sacrifice and extended maternal toxicity investigations. Final report: 12 May 2010.
- Modified prenatal developmental toxicity study in Wistar rats with epoxiconazole treatment. Final report: 12 May 2010.
- Plasmakinetic and metabolism study in pregnant rats. Final report: not determined.
- Modified maternal toxicity study in guinea pigs. Final report: 31 December 2010.
- Prenatal developmental toxicity study in guinea pigs. Final report: 30 June 2011.
- Peri-postnatal reproduction toxicology study in guinea pigs. Final report: 31 July 2011.

However, RAC was tasked only with providing an assessment of the proposal from Sweden and data gathered during the public consultation.
Consequently, in accordance with RAC procedures, RAC did not take into account of these studies, given that the information about them was presented after the public consultation has ended.

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 CLP 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:

With inclusion of classification T; R41 the use of S1/2, S45 and S53 is obligatory. S46 – *If swallowed, seek medical advice immediately and show this container or label*- is redundant with S45 - *In case of accident or if you feel unwell seek medical advice immediately (show the label where possible*- and S36/37 – *Wear suitable protective clothing and gloves* - is redundant with S53 – *Avoid exposure- Obtain special instructions before use.* S45 and S53 are considered more appropriate as these S phrases are specifically required for CMR 1 and 2.

It is therefore proposed to add S1, S45 and S53 and to remove S36/37 and S46 from the current labelling of epoxiconazole in Table 3.2 as following:

T ~~Xn~~; N
R: 40-61-62-~~63-~~51/53
S: (1/2)~~36/37-~~ 45~~-46-~~53-61

Labelling of epoxiconazole in Table 3.1 is proposed as following:

Signal word: Dgr
Pictograms: GHS08, GHS09
Hazard statements: H360Df, H351, H411

JUSTIFICATION

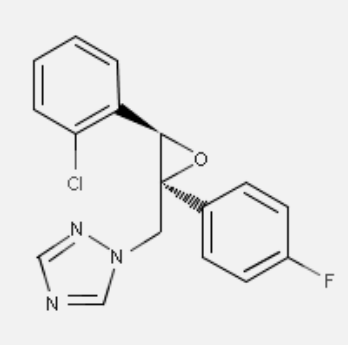
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

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

Epoxiconazole is currently classified in Annex VI of CLP under index no.: 613-175-00-9 as follows:

Carc. Cat. 3; R40

Repr. Cat. 3; R62-63

N; R51-53

For developmental toxicity, epoxiconazole 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.

The following two studies have become available since the Draft Assessment Report was finalised under Directive 91/414/EC and since the present classification was agreed on, and were evaluated by Germany, the Rapporteur Member State:

- 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.
- 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.

They concluded that based on the new data, there is no need for revising C&L. Their position was included as an addendum to the DAR in February 2008 (reference 9) but was not peer-reviewed.

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 and the two new studies available at this date were submitted. Only four countries voted for a re-opening and it was concluded by TC C&L that it was not sufficient to justify re-opening.

Sweden has submitted to ECHA in January 2009 a proposal to revise the classification of epoxiconazole for effects on development from Repr. Cat. 3; R63 to Repr. Cat. 2; R61. The two new published studies Birkhøj Kjaerstad 2007 and Taxvig 2007 not previously assessed in the 29th ATP were presented.
Besides, it was raised in the comments of the public consultation that an additional scientific paper has been published on reprotoxicity of epoxiconazole, which was not included in the Annex XV dossier of Sweden:

- Taxvig, C., Vinggaard, A. M., Hass, U., Axelstad, M., Metzdorff, S., Nellemann, C. 2008 Endocrine-disrupting properties in vivo of widely used azole fungicides. International Journal of Andrology 31, 170-177

It has been included in the Background Document.

It should also be noted that additional information was provided by Ulla Hass (see Appendix I), one the author of Taxvig studies at RAC 9 meeting and was included in the Background Document.

Moreover two more published studies (Tiboni 2009, Albrecht 2000) which were presented by an advisor of a RAC member at RAC 9 were also considered as additional elements and included in the scientific justification.

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	4000 ppm 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 1000 ppm and above Clinical chemistry indicating liver toxicity. Increased liver weights, with hepatocellular hypertrophy. 250 ppm and above 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	6400 ppm Mortality, clinical signs, body weight loss 3200 ppm and above Reduced feed consumption, clinical signs (vomiting) 1600 ppm and above Altered clinico-chemical parameters in males 400 ppm and above 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	1500 ppm Males and females: reduced food consumption and body weight gain, increased liver weights 750 ppm and higher 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 150 ppm and higher 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	1500 ppm 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 750 ppm and higher 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 150 ppm and higher 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),	1000 ppm 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 500 ppm 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):

Fertility/Developmental toxicity:

- Hellwig & Hildebrand, 1989 (oral, rat), p. 27ff
- Hellwig & Hildebrand, 1990b (oral, rat), p. 31ff
- Hellwig & Hildebrand, 1992 (oral, rat), p. 34ff
- Schneider *et al*, 2002 (oral, rat), p. 40ff
- Hellwig & Hildebrand, 1990a (oral, rabbit), p. 55ff
- Hellwig & Hildebrand, 1993 (dermal, rat), p. 58ff

Endocrine disruption:

- Mellert, 1992 (*in vivo*, rat), p. 60ff
- Wuttke, 1995 (*in vitro*), p. 66ff
- Wuttke, 2001 (*in vitro*), p. 69ff

Maternal toxicity:

- Schneider *et al*, 2001, p. 75ff

New studies not included in the classification decision process of 2003 leading to inclusion in ATP 29 but included in the Final addendum to the DAR:

Fertility/Developmental toxicity:

- Taxvig *et al*, 2007 (oral, rat), p. 47ff

Endocrine disruption:

- Taxvig *et al*, 2007 (*in vivo*, rat), p. 47ff
- Birkhøj Kjaerstad *et al*, 2007 (*in vitro*), p. 72ff

New study not included in the classification decision process of 2003, in the Final addendum to the DAR or submitted to TC C&L in 2007:

Fertility/Developmental toxicity:

- Taxvig *et al*, 2008 (oral, rat), p. 52ff

Endocrine disruption:

- Taxvig *et al*, 2008 (*in vivo*, rat), p. 52ff

Summary table of all studies on p. 77ff

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 VI of CLP and no change to 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

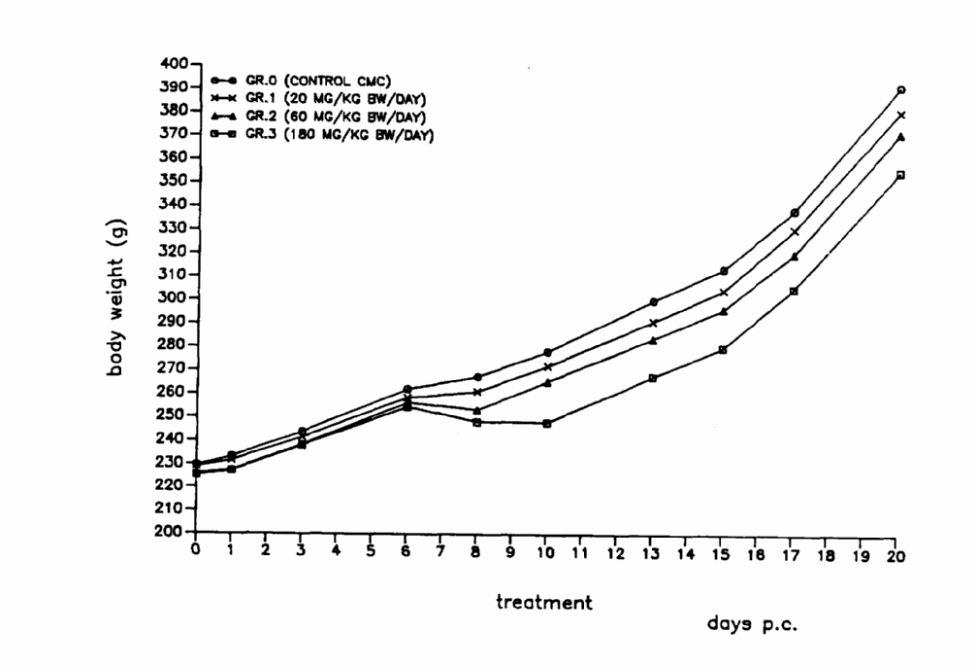
Food consumption and body weight gain were reduced during the treatment period (see Table 7 and Figure 1). At the mid and high dose levels an initial body weight loss was observed in addition to reduced weight gains during and following the treatment period. A reduced corrected body weight gain from 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 (8%)	2 (9%)	4 (20%)	7 (30%)
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)**
Cleft palate (foetal incidence, %)	0	1 (0.4%)	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/day)[$]	days 6-15	26.0	25.5 (-2%)	25.6 (-2%)	24.1 (-7%)
	days 6-8	23.8	23.6 (-1%)	23.6 (-1%)	20.8** (-13%)
	days 13-15	28.1	27.5 (-2%)	27.3 (-3%)	26.1* (-7%)
Body weight day 20 p.c. (g)		389.5	399.6	401.5	393.1
Body weight gain (g) during days 6-8[$]		6.8	5.7 (-16%)	5.4 (-21%)	1.2** (-82%)
Corrected (net) body weight gain from day 6 until termination (g)		44.1	43.8 (-1%)	44.5 (+1%)	43.6 (-1%)

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

The number of late resorptions was slightly but 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 14^{th} rib(s) at the high dose level. The concomitant reduction of foetuses with short 13^{th} 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 (19%)	4 (17%)	3 (13%)	4 (18%)
Late postimpl. loss (> 0)		3	3	5	10
Total resorptions, mean (%)[a]		1.2 (8.3%)	0.9 (5.6%)	1.1 (6.7%)	1.9 (11.8%)
Early resorptions, mean (%)		1.0 (7.3%)	0.8 (4.5%)	0.8 (5.0%)	1.3 (7.4%)
Late resorptions, mean (%)		0.1 (1.0%)	0.2 (1.1%)	0.3 (1.7%)	0.6* (4.4%)
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)
Cleft palate: foetal incidence n (%)[b]		0	0	1 (0.3%)	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 (foetal %)[c]		6(6.3%)/9	2(1.1%)/2	8(7.6%)/13	13(15.9%)/25*
Short 13th ribs, litters/foetuses (foetal %)		6(10.4%)/15	8(10.6%)/20	6(8.8%)/15	3(3.8%)/6
14th rib rudiments, litters/foetuses (foetal %)[d]		0	1(1.1%)/2	6*(4.1%)/7	15*(24.8%)/39
Total retardations: Foetal incidence n (%)		64 (23.3)	83 (22.4)	79 (23.8)	77 (25.9)

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

[a] historical control data (%): mean 7.8%; range 4.5-10.3%

[b] historical control data (foetal incidence in %): mean 0.06%; range 0-0.2%

[c] historical control data (foetal incidence in %): mean 3.7%; range 2.5-6.3%

[d] historical control data (foetal incidence in %): mean 0.8%; range 0-1.7%

Conclusion

Epoxiconazole caused transient reduced food consumption and transient 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:

<u>Parental animals:</u>

Food consumption was reduced in F0 females during lactation of F1a litters (-13%, not significant), 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 (-6% during GD14-20, $p<0.05$ and -12% during lactation, not significant). 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 (Table 12) 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.

Table 12 Fertility indices

Group affected	Dose level (ppm)			
	0 (control)	10	25	250
Male fertility index				
F1a mating	96%	96%	96%	88%
F1b mating	96%	100%	92%	78%
F2 mating	100%	88%	100%	84%*
Female fertility index				
F1a mating	96%	96%	96%	88%
F1b mating	96%	100%	92%	78%
F2 mating	100%	88%	100%	88%

* $p<0.05$

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 13. 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 (-13%, not significant); F1 males especially at the beginning of pre-mating period; F1 females during pregnancy (-6%, $p<0.05$) and lactation (-12%, not significant)
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 but outside historical control values in F2 only. 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 F1b, 1 foetus (0.4%) exhibited anasarca. In three litters of F2 one pup each exhibited anasarca (generalised oedema) at birth (1.3%). 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) and above historical control range in F2 (historical control mean: 0.03%, range: 0-0.3%).

One foetus (0.4%) with a cleft palate was reported in F1b (historical control mean: 0.03%, range: 0-0.3%).

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 14. 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 (%)[a]				
F1a	90	90	92	93
F1b	95	94	93	90*
F2	98	93**	94**	82**
Lactation index (%)[b]				
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)
[a] historical control range: 89-99%
[b] historical control range: 95-100%

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 but the decrease was within historical control range. The finding of anasarca 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 15. 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
Oestradiol	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 16). 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 16. 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.

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

Table 17. 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	
Oestradiol (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 18. 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 (%)[a]	0.5 (9.9%)	5.3** (59%)	4.0** (42.9%)
Early resorptions, mean (%)	0.4 (9.4%)	0.8 (8.9%)	0.5 (4.9%)
Late resorptions (mean)	0.1 (0.5%)	4.5** (50.1%)	3.5** (38.0%)
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

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

[a] historical control mean: 7.1%; range 3.8-11.3%

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 19 and 20).

Table 19. 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 (6%, statistically significantly increased) and two batch #2 foetuses (1.7%, n.s.). This malformation has never been reported in the historical control data (0/1495). 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 (2.4%) and one foetus of batch #2 (0.8%). This malformation has never been reported in the historical control data (0/1495). 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 20).

Table 20. 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 containing higher impurity content. 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. 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. In contrast, cleft palate, present with a high prevalence in the dose-finding study, was only observed in one litter each. In this study, higher post-implantation loss may however have masked teratogenic effects. 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.

(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 and Hass U. *2010*, Further information in relation to the Taxvig et al 2007 and 2008 performed in our laboratory. Personal communication to the Risk Assessment Committee (included in appendix I)..

GLP: Not stated

Guideline: No

Acceptability: Acceptable/Non-guideline

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 decapitation from all foetuses for hormone analysis and one pool per litter was made for all male and female foetuses, respectively.

The remaining dams were delivered their pups (13, 10 and 3 in the control, 15 and 50 mg/kg group, 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 oestradiol 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. Hormonal levels were also measured in dams at GED 21.

Semen quality of the sexually mature males (11-12 animals /group, 1-2 / litter) was analysed in the controls and low dose groups. None of the high dose animals was kept for this analysis due to foetal and neonatal toxicity. Animals approximately 7-month old were sacrificed and the epididymides were removed. The cauda of the left epididymis was used for motility analysis. Spermatozoa were obtained from the distal cauda and sperm sample were prepared for analysis to determine percent motile and percent progressive spermatozoa, curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH), average path velocity (VAP), straight line velocity (VSL) and straitghness (STR). Cauda of the left epididymis was frozen in liquid nitrogen and later prepared for sperm count.

Further investigations of the individual data were provided as a personal communication further to the RAC-9 (Hass U 2010) and are included in Appendix I. Correlation between the percentages of very late resorptions in litters versus the dam progesterone level in plasma on GD 21 was determined in an attempt to investigate the mechanism of action of very late resorption and its potential link to endocrine disruption in the dams.

Findings

In the post-natal part of the study, the highest dose of epoxiconazole increased gestational length, post-implantation 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. Perinatal deaths were also reported. They are defined as the number of live pups at weaning relative to the number of implantation (Hass, 2010). They therefore account for post-implantation loss as well as postnatal deaths. These findings are however based on a very limited number of litters (n=3).

In dams submitted to Caesarean section, an increase of post-implantation loss was also observed and 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. However, in the group of dams allowed to deliver, a tendency towards a decreased maternal weight gain GD7-PND1 was observed and a tendency towards a decreased body weight gain PND1-PND13 was observed at 15 mg/kg but not at 50 mg/kg although data are based on only one animal at this dose. Birth weight of offspring was statistically significantly increased from 15 mg/kg

whereas, in the caesarian section on GD 21, a decrease in foetal weight in both sexes was seen in the highest dose group.

Main findings as published in Taxvig 2007 are summarised in Table 21.

Table 21. Pregnancy and Litter Data as in Taxvig 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.

Very late resorptions were defined as dead foetus with small or no external degenerative changes, developed at least to GD15. In the OECD 414, the term for this appear to be dead foetus (Hass 2010). Very late resorptions were calculated as a percentage of late resorptions. Calculations with each type of resorptions related to the total number of implantations were considered to be more in line with OECD guidelines calculations and are given in Table 22 according to information provided by the author (Hass, personal communication). Statistical significance was not provided.

Table 22 Resorptions by types calculated based on total number of implantations

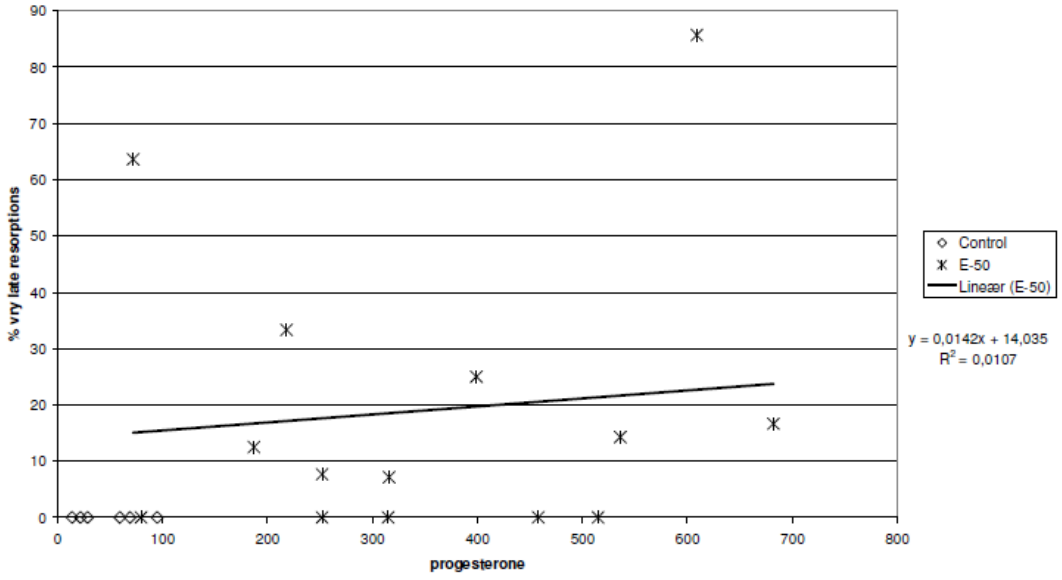
Type of resorptions	Doses (mg/kg/d)		
	0	15	50
Early resorptions (%)	5.2±6.6	7.0±8.4	3.0±6.4
Late resorptions (%)	1.3±3.1	10.2±22.7	5.8±13.6
Very late resorptions (%)	0.0±0.0	4.2±11.8	19.0±26.1
Total resorptions (%)	6.5±7.9	20.9±33.5	27.9±28.8

Anogenital distance was increased both in female foetuses at GD 21 and in newborn female offspring although relevant data are not available at the high dose. In male foetuses at GD 21, a significantly increased AGD was seen when dividing the AGD with the cubic-root of the body weight only at the low dose. In male offspring at PND 16, no effects on AGD were seen.

No significant effect on the number of aerolas was observed in males and females at PND 16.

The percentage of very late resorptions in litters versus the dam progesterone level in plasma on GD 21 is shown in Figure 2. No correlation was identified between the two endpoints (R^2 = 0.01).

Figure 2 % very late resorptions vs progesterone in dam plasma GD21 (Taxvig 2007)



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 oestradiol levels in ovaries of 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 (see Table 23).

Table 23 Hormonal levels

Hormone	Doses (mg/kg)		
Dams at GD 21	0	15	50
Testosterone (nM)	0.40±0.25	0.49±0.31	**0.82±0.43***
Progesterone (nM)	48±32	170±141	**349±190***
Male foetuses at GD21	0	15	50
17α –OH-progesterone (ng/testis)	1.95±0.54	1.76±1.36	0.94±0.48
Testosterone (ng/testis)	1.75±0.71	1.62±0.59	1.11±0.56
Progesterone (ng/testis)	0.037±0.025	0.029±0.019	0.027±0.019
Male pups at PND16	0	15	50
Testosterone (ng/ml)	0.14±0.18	0.07±0.14	0.02
Female pups at PND 16	0	15	50
Oestradiol (ng/ml)	8.40±3.90	5.00±2.70	3.6

On PND 16, a tendency (not significant) towards increased liver weight and decreased adrenals weight in offspring was seen in the highest dose group. There was also a tendency towards an increased weight of all male reproductive organs, without any effects on body weight at the highest dose although a tendency for a decrease was observed at the low dose. Besides, 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.

In sexually mature male offspring (7 months), the group dosed with 15 mg/kg had three males out 12 with severely reduced sperm number. Sperm quality could not be analysed for these animals and they were therefore excluded from the statistical analysis to enable its determination. No statistically significant reduction in the number of sperm cells was observed and no statistically significant reductions were found on sperm motility or velocity parameters compared to control group.

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. The increased gestational length is probably due to the increased levels of progesterone that was seen in the dams.

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

Report: Taxvig C *et al*. 2008, Endocrine-disrupting properties in vivo of widely used azole fungicides. International Journal of Andrology 31, 170-177 and Hass U *2010*, Further information in relation to the Taxvig et al 2007 and 2008 performed in our laboratory. Personal communication to the Risk Assessment Committee (included in appendix I).

GLP: Not stated

Guideline: No

Acceptability: Acceptable/Non-guideline

For further details, Taxvig *et al*, 2008.

Material and methods

Test material: Pure epoxiconazole (99%) from Dr Ehrenstorfer (Augsburg, Germany). As vehicle, corn oil from Sigma-Aldrich (Brønby, Denmark) was used.

Test animals: 50 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 ketoconazole, propiconazole and tebuconazole. The day after arrival (GD4), animals were weighed and assigned to five groups of 10 animals (including three groups that were administered ketoconazole, propiconazole or tebuconazole; results will not be presented here). The two groups that were included in the epoxiconazole testing consisted of 10 animals each (n=20), with similar weight distribution. Epoxiconazole was administered by oral gavage at doses of 0 (control) or 50 mg/kg bw from GD 7 until GD21. Of the time-mated animals, 6 and 8 in the control 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.

Caesarian sections were performed on GD 21. 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 decapitation from all foetuses for hormone analysis and one pool per litter was made for all male and female foetuses, respectively. In all live pups anogenital distance (AGD) was measured.

At GD 21 levels of testosterone and progesterone were analysed in serum from the dams and fetuses and 17α-hydroxyprogesterone in the dams. In fetuses, steroid hormones levels were analysed in testis and oestradiol was analysed in ovaries.

Further investigations of the individual data were provided as a personal communication further to the RAC-9 (Hass U 2010). Correlation between the percentages of very late resorptions in litters versus the dam progesterone level in plasma on GD 21 was determined in an attempt to investigate

the mechanism of action of very late resorption and its potential link to endocrine disruption in the dams.

Findings

Epoxiconazole induced a high frequency of post-implantation loss, a marked increase in late and very late resorptions and an increase in fetal weight in both sexes. No significant effects were seen on AGD although a non significant decrease is seen in female fetuses.

Main findings as published in Taxvig 2008 are summarised in Table 24.

Table 24. Pregnancy and Litter Data as in Taxvig 2008

GD21 Caesarian section	1. Control	4. Epoxi-50
No. dams	6	8
Maternal body weight	224.0 ± 8.3	218.2 ± 3.9
No. implantations	11.3 ± 0.8	11.9 ± 0.5
No. live fetuses	11.3 ± 0.8	9.0 ± 1.1
% post-implantation loss	0.0 ± 0.0	**25.1 ± 8.0** *
% late res	0.0 ± 0.0	**22.4 ± 7.9** *
% very late res	0.0 ± 0.0	**15.5 ± 5.6** *
% males	56.5 ± 2.6	61.1 ± 6.3
Male fetal weight[a]	3.6 ± 0.2	**4.8 ± 0.3***
Female fetal weight[a]	3.5 ± 0.2	**4.3 ± 0.3***
Male AGD (mm)[b]	3.76 ± 0.08	3.97 ± 0.06
Male AGD index	2.47 ± 0.03	2.39 ± 0.04
Female AGD (mm)[b]	2.12 ± 0.03	2.07 ± 0.04
Female AGD index	1.40 ± 0.03	1.28 ± 0.03

Data represent group means based on litter means ± SEM. Epoxi-50 = 50 mg/kg bw/day epoxiconazole. Values shown in bold are statistically significantly different compared to control, * p < 0.0.5.
[a]Fetal body weight was calculated using the live number of fetuses as covariate.
[b]AGDs was analysed both with and without the cubic root of body weight as a covariate and both analyses showed Epoxiconazole group was not significantly affected. AGD index means the AGD divided by the cubic root of body weight.

Very late resorptions were defined as dead foetus with small or no external degenerative changes, developed at least to GD15. In the OECD 414, the term for this appear to be dead foetus (Hass 2010). Very late resorptions were calculated as a percentage of late resorptions. Calculations with each type of resorptions related to the total number of implantations were considered to be more in line with OECD guidelines calculations and are given in Table 25 according to information provided by the author (Hass, personal communication). Statistical significance was not provided.

Table 25 Resorptions by types calculated based on total number of implantations

Type of resorptions	Doses (mg/kg/d)	
	0	50
Early resorptions (%)	0.0±0.0	2.68±7.58
Late resorptions (%)	0.0±0.0	6.95±8.42
Very late resorptions (%)	0.0±0.0	15.46±15.94
Total resorptions (%)	0.0±0.0	25.09±22.55

In dams, a decrease in the plasma oestradiol level and an increase in testosterone level were seen in the epoxiconazole group but no significant effect on progesterone levels. No significant effects were found on the measured hormone levels in fetuses (data not shown for fetal plasma testosterone and progesterone levels and oetradiol level in ovaries). However, a tendency towards a decrease in testosterone production was seen.

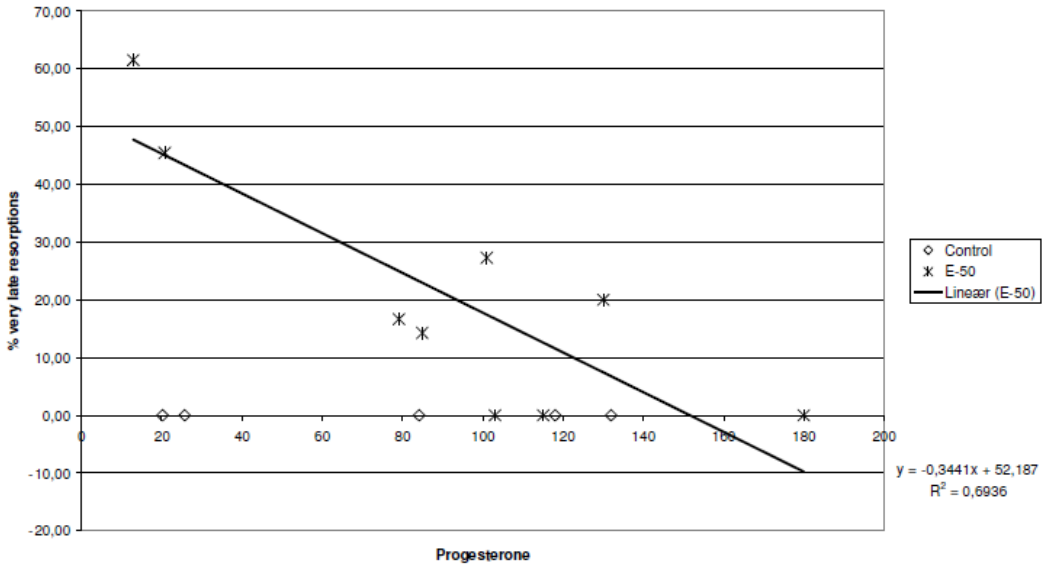
Table 26. Plasma hormone levels in dams at GD21 and testicular hormone levels in fetuses (as in Taxvig *et al*, 2008)

	17α-Hydroxyprogesterone (ng/mL)	**Progesterone** mean (nm)	**Testosterone** mean (nm)	**Oestradiol** mean (nm)
Control	13.11 ± 4.02 (8)	95 ± 100 (9)	0.38 ± 0.32 (9)	0.036 ± 0.024(9)
Epoxyconazole 50 mg/kg	23.77 ± 10.41 (9)	175 ± 102 (10)	**0.99 ± 0.57*(10)**	**0.021 ± 0.006*(10)**
	Progesterone (ng/testis)	**Testosterone (ng/testis)**	**Testosterone production (ng/testis)**	**Progesterone production (ng/testis)**
Control	0.08 ± 0.04 (16)	1.64 ± 0.68 (13)	4.90 ± 3.80 (6)	0.001 ± 0.00 (6)
Epoxyconazole 50 mg/kg	0.06 ± 0.04 (16)	1.42 ± 0.81 (17)	2.40 ± 1.10 (8)	0.003 ± 0.003 (8)

Data represent mean ± SD. Values shown in bold are statistically significantly different compared to control, * $p < 0.0.5$. ( ) = n.

The percentage of very late resorptions in litters versus the dam progesterone level in plasma on GD 21 is shown in Figure 3. A negative correlation was identified between the two endpoints (R^2 = 0.69). However, this result may be coincidental as maternal progesterone levels were not significantly altered in this study.

Figure 3 % very late resorptions vs progesterone in dam plasma GD21 (Taxvig 2008)



Conclusion

Epoxiconazole had a marked foetotoxic effect. No demasculinizing effects were seen in male fetuses and no virilising effects in female fetuses. In contrast, an increased fetal weight was observed, which might be related to the up-regulated levels of testosterone in the dams.

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 27. 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**(-16%)	79.0* (-13%)	79.1* (-13%)
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 (-44%)	- 131 (-17%)	- 125 (-13%)
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. One cleft palate was observed at the lowest dose but in the absence of such findings at the mid- and high-doses, its significance is unclear.

Table 28. 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 (%)[a]	13.6%	2.3%	10.2%	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, mean (%)	0.2 (3.2%)	0.0	0.1 (2.2%)	0.2 (2.2%)
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 (%)	3 (4%)	3 (3.2%)	0 (0.0%)	0 (0.0%)
Cleft palate : foetal incidence n (%)	0	1 (1.1%)	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)**

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

[a] Historical controls range: 4.9-18.4%

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 29. 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 %)[a]	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 (2%)	0.3 (2%)	0.1 (0.7%)	0.2 (1.4%)
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
Cleft palate: foetal incidence (%)[b]	0	0	0	1 (0.3%)
Cervical ribs (litters/foetuses)	1/2	2/2	5/8	9/12
14th ribs (litters/foetuses)	1/1	1/1	4/6	7/10

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

[a] Historical controls range: 4.5-15.7%

[b] Historical controls mean: 0.03%; range: 0-0.4%

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

No data available.

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 30.

Table 30. 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 31.

Table 31. 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 32. 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
Oestradiol	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 33. 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
Oestradiol	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 oestradiol, was seen although not being statistically significant (see Table 32). 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 33), or a trend, which was considered to be test substance-related.

Table 34 shows further hormone alterations due to the administration of the test substance to males at 3000 ppm for 4 weeks.

Table 34. Hormone level alterations in males after a 4-week treatment with 3000 ppm (% of control)

Testosterone	124 %
Androstenedione	170 % *
Oestradiol	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 35), significantly increase levels of dehydroepiandrosterone, androstendione, LH and FSH were seen together with a decrease in oestradiol and corticosterone, although the latter not being statistically significant. After 4 weeks, changes in the same hormones could be seen, the increase in oestradiol 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 35. 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***
Oestradiol	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 36), changes in the same hormones as during dioestrus was seen, but the decrease in oestradiol 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 36. 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
Oestradiol	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 37 shows further hormone alterations due to the administration of the test substance to females at 3000 ppm for 4 weeks.

Table 37. Changes of hormone values in high dose females (% of control value)

Hormone	Dioestrus	Prooestrus
Dehydroepiandrosterone	270***	150**
Androstenedione	470***	232
Oestradiol	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 oestradiol was decreased and LH and FSH were upregulated, especially during dioestrus. Exposure at 1500 ppm was sufficient to reduce oestradiol 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, oestradiol, 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 oestradiol. The inhibition of aromatase leads to an increased concentration of androgens and a decreased concentration of oestradiol. The decreased oestradiol 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 oestradiol, 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.

Oestradiol 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 oestradiol 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 38, 39 and 40 are approximations and not exact figures.

Table 38. Effects on oestradiol production

Epoxiconazole concentration (µmol/L)	Oestradiol (% 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 oestradiol 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 oestradiol production was reduced by approximately 70 %. In human granulosa cells a concentration of 1 µmol/L was needed to inhibit oestradiol 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 39. 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 40. 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 oestradiol, 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 4a. Influence of vorozole on the aromatase activity of human granulosa cells (as in Wuttke, 2001)

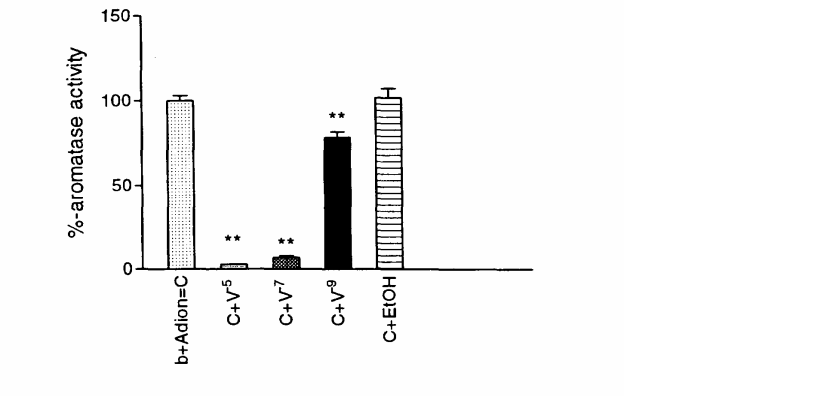


Figure 4b. Influence of vorozole on the aromatase activity of rat granulosa cells (as in Wuttke, 2001)

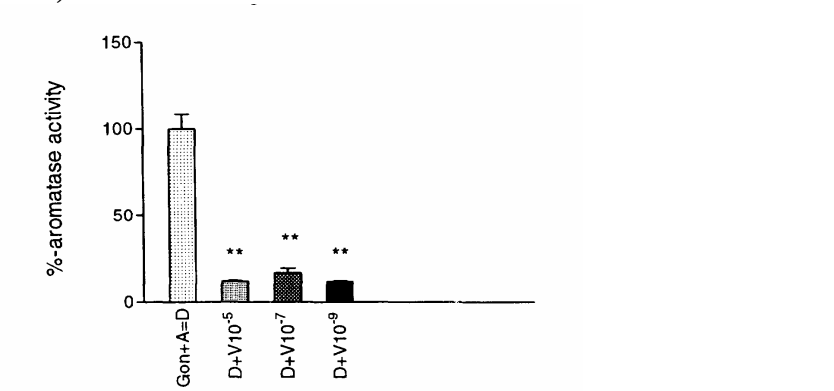


Fig.4a, b. Human and rat granulosa cells were incubated with vorozole (V) for 48 h under standard conditions. For the determination of the aromatase activity in human granulosa cells (fig 2a), 4-androstenedione (10 nM) was added to all experimental preparations. The influence of the solvent, ethanol, 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 5a. Influence of epoxiconazole on the aromatase activity of human granulosa cells (as in Wuttke, 2001)

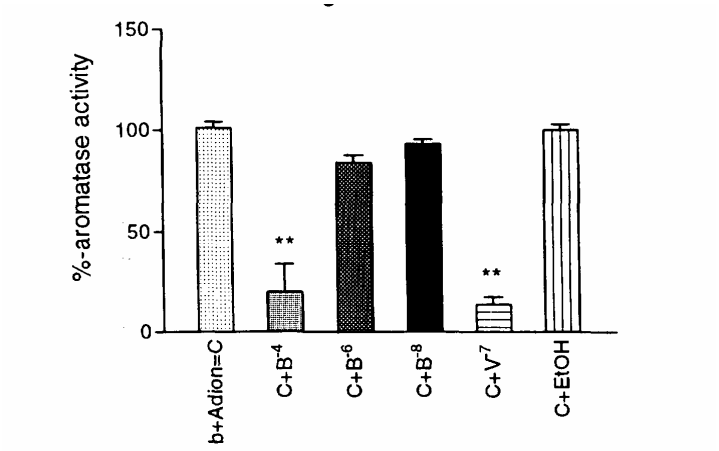


Figure 5b. Influence of epoxiconazole on aromatase activity of rat granulosa cells (as in Wuttke, 2001)

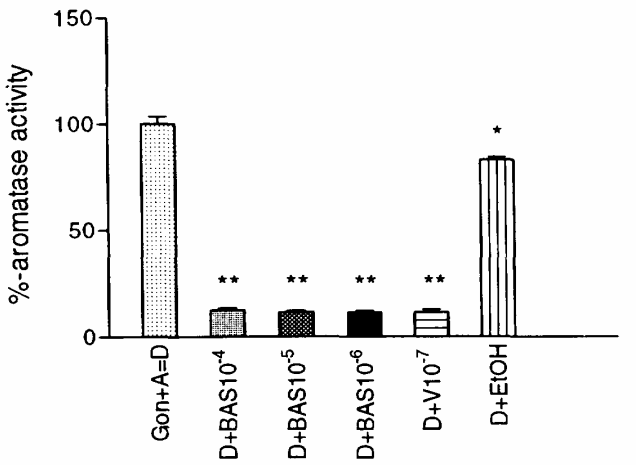


Fig.5a,b. Human granulosa cells (fig 3a) were incubated with epoxiconazole (B) for 48 h under standard conditions. For the determination of the aromatase activity, 4-androstenedione (10 nM) was added to all experimental preparations. The influence of the solvent, ethanol, was also investigated (C+EtOH). 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

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 oestradiol), 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-oestradiol, 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 = 73µM). The concentrations needed to reduce the testosterone induced response were lower than the concentrations needed to reduce 17β-oestradiol, 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 oestradiol 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 oestradiol.

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

See 5.9.2 Developmental toxicity

Report: Taxvig C *et al*. 2008, Endocrine-disrupting properties in vivo of widely used azole fungicides. International Journal of Andrology 31, 170-177.

See 5.9.2 Developmental toxicity

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 41), 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 41. 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 42. 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 of reproductive toxicity

Data on reproductive and developmental toxicity and endocrine disruption studies are summarised in Table 43.

Table 43. Epoxiconazole - summary table of reproductive and developmental toxicity

Author(s)/Year	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
RAT - ORAL EXPOSURE		
Hellwig J. Hildebrand B. 1989	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	Dams 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 Foetuses 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.) 1 cleft palate (0.4%) at 20 mg/kg.
Hellwig J., Hildebrand B.; 1990b	Prenatal toxicity, gavage, Chbb:THOM (SPF) Wistar rats: 0, 5, 15, 45 mg/kg bw on days 6 – 15 p.c., acceptable	Dams 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 Foetuses 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) 15 mg/kg : 1 cleft palate (0.3%)
Hellwig J. Hildebrand B. 1992	Two-generation oral feeding, Chbb:THOM (SPF) Wistar rats, 0, 10, 25, 250 ppm, acceptable	Parents, 250 ppm: 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) Liver weights increased in F1 females without

Author(s)/Year	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
		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 cleft palate: F1b: 1 pup body wt gain and body wt decreased
Schneider S. *et al*. 2002	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) Oestradiol, 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, cleft palate in 2 and 1 foetuses 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	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
		Foetuses/pups Increased post-implantation loss of foetuses (mainly very late resorptions) and postnatal death of pups Incidence of stillbirths increased, live litter size decreased Increased anogenital distance in female foetuses and offspring
Taxvig *et al.* 2008	*In vivo* investigation of endocrine disrupting activities, acceptable	Dams Levels of testosterone increased and of oestradiol decreased Foetuses/pups Increased post-implantation loss, late resorptions and fetal weights. No significant effect on anogenital distance or hormonal levels in foetuses.
RAT - DERMAL APPLICATION		
Hellwig J., Hildebrand B. 1993	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	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, (Interim report) and Mellert W., Hildebrand B. 1999, (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

Author(s)/Year	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
		1500 ppm and above Males and females: reduced food consumption Males: increased androgen and FSH levels Females: increased androgen levels, prolonged oestrous cycles, decreased oestradiol levels, decreased aldosterone and prolactin levels during prooestrus
Wuttke W. 1995	*In vitro* investigations of effects on ovarian, adrenal and pituitary hormones, different cell types (rat, pig, human) 0.01 – 10 µmol/L, acceptable	Aromatase inhibition Porcine luteal cells > rat granulosa cells > human granulosa cells Progesterone production 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	*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 oestradiol 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	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

Author(s)/Year	Study type, Species/strain, Dose levels (vehicle), Acceptability	Findings/Comments
		decreased, aspartate amino transferase and glucose levels increased

5.9.6 Discussion of reproductive toxicity

The whole database raises questions on several aspects of developmental toxicity and each is discussed in a separate section below:

- endocrine disruption and its potential developmental effects
- post-implantation loss
- teratogenic effets and in particular induction of cleft palate
- peri- and postnatal deaths

Endocrine disruption and its developmental effects

The endocrine disruptive potential of epoxiconazole has been identified *in vitro* is several studies (Birkhøj Kjaerstad 2007, Wuttke 1995 and 2001) and consisted mainly in inhibition of the aromatase involved in the biosynthesis of estrogens from androgens.

In vivo, the main effects of epoxiconazole on hormonal level in the adult females are summarized in Table 44 below.

Table 44 Effect of epoxiconazole on hormonal level in female adult rats

Study/Hormone measured	Doses			Comment
Mellert 1992 and 1999	0	1500 ppm	3000 ppm	Exposure for 4 weeks, females in prooestrus
Oestradiol (pg/mL)	39±6.8	**19±7.6***	**8.1±5.3***	
Prolactine (ng/mL)	107±39	**92±32***	**4.7±6.0***	
LH (ng/mL)	5.9±3.3	10.0±8.6	2.9±0.4	
FSH (ng/mL)	17±2.0	**21±5.0***	18±1.5	
Dehydroepiandrosterone (ng/mL)	1.0±0.51	**2.1±0.51***	**1.5±0.55***	
Androstenedione (ng/mL)	0.95±0.20	**3.5±0.51***	**1.5±0.55***	
Mellert 1992 and 1999	0	1500 ppm	3000 ppm	Exposure for 4 weeks, females in dioestrus
Oestradiol (pg/mL)	22±18	4.5±4.4	**3.1±3.5***	
Prolactine (ng/mL)	1.9±1.3	2.1±1.9	2.2±1.4	
LH (ng/mL)	2.1±0.3	**3.0±0.7***	**4.7±1.6***	
FSH (ng/mL)	14±1.0	**16±1.2***	**18±2.4***	
Dehydroepiandrosterone (ng/mL)	0.48±0.18	**1.5±0.48***	**1.3±0.32***	

Androstenedione (ng/mL)	0.32±0.12	**1.5±0.52***	**1.8±0.45***	
Schneider 2002	0	180 (batch 1)	180 (batch 2)	Exposure during GD 6-19, measure on GD 20
Progesterone (nmol/L)	84.72	**17.05***	**18.22***	
Oestradiol (pmol/L)	197.89	**86.81***	**106.86***	
Prolactine (µg/L)	4.21	**2.38***	**1.35***	
LH (µg/L)	1.30	**1.93***	1.75	
Taxvig 2007	0	15	50	Exposure during GD 7-21, measure on GD 21
Testosterone (nM)	0.40±0.25	0.49±0.31	**0.82±0.43***	
Progesterone (nM)	48±32	170±141	**349±190***	
Taxvig 2008	0	-	50	Exposure during GD 7-21, measure on GD 21
Testosterone (nM)	0.38±0.32	-	**0.99±0.57***	
Progesterone (nM)	95±100	-	175±102	
Oestradiol (nM)	0.036±0.024	-	**0.021±0.006***	

Overall, this data indicate that epoxiconazole consistently induce a decrease in oestradiol in females. No consistent significant effect was seen on progesterone level.

Prolonged duration of gestation and difficulties in parturition have been observed repeatedly in the two post-natal studies available (Taxvig 2007, Hellwig 1992-two-generation study) and these effects may be consistent with the observed disturbance of hormonal levels. However, these effects are considered as effects on fertility and are not further discussed here.

Regarding developmental effects, the Taxvig studies were designed to investigate specifically potential endocrine disruptive effects in the offspring further to *in utero* exposure to epoxiconazole.

In these studies no clear significant effect was seen on hormonal level in reproductive organs of either males and females foetuses and pups exposed during gestation as seen in Table 45 (males) and 46 (females).

Table 45 Effect of epoxiconazole on hormonal levels in male rat foetuses and pups

Study/Hormone measured	Doses (mg/kg)			Comment
Taxvig 2007	0	15	50	
17α –OH-progesterone (ng/testis)	1.95±0.54	1.76±1.36	0.94±0.48	Exposure during GD 7-21, measure on GD 21
Testosterone (ng/testis)	1.75±0.71	1.62±0.59	1.11±0.56	

Progesterone (ng/testis)	0.037±0.025	0.029±0.019	0.027±0.019	
Taxvig 2007	0	15	50	
Testosterone (ng/ml)	0.14±0.18	0.07±0.14	0.02	Exposure during GD 7 to PND 16, measure on PND16
Taxvig 2008	0	-	50	
Testosterone (ng/testis)	1.64±0.68	-	1.42±0.81	Exposure during GD 7-21, measure on GD 21
Progesterone (ng/testis)	0.08±0.04	-	0.06±0.04	

In males, a tendency to a decrease in testosterone and in progesterone was seen in both Taxvig studies but without statistical significance.

Table 46 Effect of epoxiconazole on hormonal levels in female rat foetuses and pups

Study/Hormone measured	Doses (mg/kg)			Comment
Taxvig 2007	0	15	50	
Oestradiol (ng/ml)	8.40±3.90	5.00±2.70	3.6	Exposure during GD 7 to PND 16, measure on PND16
Taxvig 2008	0	-	50	
Oestradiol	No variation. Data not shown.			Exposure during GD 7-21, measure on GD 21

In females, a tendency to a decrease in oestradiol level was seen in Taxvig 2007 but not statistical significance and not repeated in Taxvig 2008.

The anogenital distance (AGD) in offspring was also measured (Table 47) and AGD index was calculated to take into account offspring body weight.

Table 47 Effect of epoxiconazole on AGD index in rat foetuses and pups

Study/Hormone measured	Doses (mg/kg)			Comment
Taxvig 2007	0	15	50	
Male AGD index	2.08±0.1	**2.31±0.1***	2.25±0.1	Exposure during GD 7-21, measure on GD 21
Female AGD index	1.04±0.1	**1.28±0.2***	**1.29±0.1***	

Taxvig 2007	0	15	50	
Male AGD index	1.92±0.1	1.96±0.1	1.83±0.2	
Female AGD index	0.98±0.03	**1.08±0.1***	0.96	Exposure during GD 7 to PND 16, measure on PND16
Taxvig 2008	0	-	50	
Male AGD index	2.47±0.03	-	2.39±0.04	Male AGD index
Female AGD index	1.40±0.03	-	1.28±0.03	Female AGD index

In males, a statistically significant increase in AGD index is seen at the low dose in Taxvig 2007 on GD21 but not at the higher dose. Aa absence of dose-response and significance is also observed at PND16. A tendency to a decrease without statistical significance is seen in Taxvig 2008.

In females, a statistically significant increase in AGD index is observed in Taxvig 2007 both at GD21 and PND16 but a tendency to a decrease is seen in Taxvig 2008 (not significant).

Overall, no significant, reproducible effect of epoxiconazole on offspring AGD is identified.

The number of aerolas measured at PND16 in Taxvig 2007 was not statistically significantly different between controls and treated groups for both males and females pups.

Considering potential effect of epoxiconazole on reproductive function of offspring via endocrine disruptive effects, an increase in the weight of reproductive organs was generally seen in males pups on PND16 at the high dose (Taxvig 2007). However, the effect was statistically significant for ventral prostate only and was based on a very limited number of animals and a decrease was generally seen at the low dose. Besides, at histological examination that was performed on low dose animals only, adverse effect in the testis (absence of germ cell) were seen in only one animal out of 29. In this study, animals were also examined at 7 months for sperm count and motility. No effect was reported although 3 outliers with no motile sperm out of 12 animals were excluded from analysis.

The two-generation study also brings valuable information on animals that are exposed *in utero* to epoxiconazole. At the highest dose of approximately 23 mg/kg/d an increase in the time to mating was seen in several animals and fertility indices were generally reduced for males and females. However, all animals were proved to be fertile and these effects were seen in a similar magnitude than with previous generation and it cannot be attributed to *in utero* exposure of F1a animals. Besides, no effect was seen in the histopathological examination of reproductive organs of either F1a parents or F2 pups.

Overall, although an endocrine disruptive potential has been identified *in vitro*, no significant reproducible developmental effect has been identified in the offspring exposed *in utero* based on available data.

Post-implantation loss

Several prenatal developmental toxicity studies are available and provide information on the induction of post-implantation loss. Data from the two-generation study are also relevant. Data on post-implantation loss and level of general maternal toxicity such as effect on maternal body weight or food consumption are summarised in Table 48 below.

Table 48 Summary of information on post-implantation loss.

Hellwig 1989	Dose (mg/kg/d)	0	20	60	180
OCDE 414 range-finding Oral Wistar rat (n=25) GD6-15	Maternal corrected bw gain	-	**-16%***	**-24%***	**-49%***
	Maternal food conso GD6-15	-	-6%	-13%	**-27%***
	Post-implantation loss (>2 implants)	2/24 (8%)	2/23 (9%)	4/20 (20%)	7/23 (30%)
	Mean total resorptions	1.3	0.9	1.9	2.9
	Mean late resorptions	0.3	0.3	0.9	0.9
Hellwig 1990b	Dose (mg/kg/d)	0	5	15	45
OCDE 414 Oral Wistar rat (n=25) GD6-15	Maternal corrected bw gain	-	-1%	+1%	-1% (transient bwg ¬ GD6-8)
	Maternal food conso GD6-15	-	-2%	-2%	-7%
	Maternal food conso GD6-8	-	-1%	-1%	**-13%***
	Maternal food conso GD13-15	-	-2%	-3%	**-7%***
	% post-implantation loss	8.3%	5.6%	6.7%	11.8%
	Mean total resorptions (%)	1.2 (8.3%)	0.9 (5.6%)	1.1 (6.7%)	1.9 (11.8%)
	Mean late resorptions (%)	0.1 (1.0%)	0.2 (1.1%)	0.3 (1.7%)	**0.6* (4.4%)***
Schneider 2002	Dose (mg/kg/d)	0	180 (batch 1)	180 (batch 2)	
OCDE 414 with a single dose Oral Wistar rat (n=25) GD6-19	Maternal corrected bw gain	-	**-45%***	**-30%***	
	Maternal food conso GD6-19	-	-14%	-6%	
	Maternal food conso GD6-8	-	**-30%***	-6%	
	Maternal food conso GD15-20	-	**-20%**	**-17%***	
	% post-implantation loss	9.9%	**59.0%***	**42.9%***	
	Mean total resorptions	0.5	**5.3***	**4.0***	
	Mean late resorptions (%)	0.1 (0.5%)	**4.5* (50.1%)***	**3.5* (38.0%)***	
Taxvig 2007	Dose (mg/kg/d)	0	15	50	
Oral Wistar rat (n=6,9,18) GD7-GD21	Maternal corrected bw gain	-	-7%	-7%	
	Maternal food consumption	Not measured			
	% post-implantation loss	6.45%	20.87%	**28.14%***	
	% late resorptions	1.28%	10.2%	5.8%	
	% very late resorptions	0%	4.2%	**19.0%***	
Taxvig 2008	Dose (mg/kg/d)	0	-	50	
Oral Wistar rats	Maternal adjusted bw GD21	-	-	-3%	
	Maternal food consumption	Not measured			

(n=10) GD7-GD21	% post-implantation loss	0	-	**25.1%***	
	% late resorptions	0	-	**6.95%***	
	% very late resorptions	0	-	**15.5%***	
Hellwig 1993	Dose (mg/kg/d)	0	100	400	1000
OCDE 414 **Dermal** Wistar rat (n=25) GD6-15	Maternal corrected bw gain	No effect reported			
	Maternal food consumption	No effect reported			
	% post-implantation loss	8.8%	6.7%	5.9%	8.3%
	Mean total resorptions	1.3	1.0	0.8	1.2
	Mean late resorptions	0.3 (≈ 2%)	0.3(≈ 2%)	0.1(≈ 0.7%)	0.2(≈ 1.4%)
Hellwig 1990a	Dose (mg/kg/d)	0	5	20	80
OCDE 414 Oral **Rabbit** (n=15) GD7-19	Maternal corrected bw gain	-	-44%	-17%	-13%
	Maternal food conso GD7-19	-	**-16%***	**-13%***	**-13%***
	% post-implantation loss	13.6%	2.3%	10.2%	**43.0%***
	Mean total resorptions	0.9	0.14	0.5	**3.3***
	Mean late resorptions (%)	0.2 (3.2%)	0	0.1 (2.2%)	0.2 (2.2%)
Hellwig 1992	Dose (mg/kg/d)	0	~ 0.9	~ 2.3	~ 23
OCDE 416 Oral Wistar Rat (n=25) 0-10-25-250 ppm	Maternal F0 food conso	-	-	-	-13% in lactation of F1a
	Maternal toxicity F0: vaginal hemorrhages	-	-	-	6 dams (F1a)
	Prolonged duration of pregnancy F1a F1b	0 3	0 1	2 4	**9*** 3
	Mean litter size F1a F1b	13.3 13.8	12.3 13.7	13.7 13.0	**9.1*** **12.2***
	Maternal F1 food conso	-	-	-	**-6%*** GD14-20 and -12% in lact.
	Maternal toxicity F1: vaginal hemorrhages	-	-	-	1 dam
	Prolonged duration pregnancy	2	0	2	**6***
	Mean litter size F2	11.7	12.9	10.8	10.9

By oral route, whereas no significant increase in post-implantation loss was observed in studies in which rats were exposed to 45 mg/kg/d epoxiconazole (Hellwig 1990b) and to 180 mg/kg/d (Hellwig 1989) from GD 6 to 15, a large increase of post-implantation loss was observed in Schneider 2002 at the same dose of 180 mg/kg/d with an exposure partially extended to the end of gestation (GD 6-19). Resorptions were mainly identified as late resorptions.
In Taxvig 2007 and 2008, in which exposure was entirely extended to the end of gestation (GD7-21), a significant increase in post-implantation loss was observed at 50 mg/kg/d and consisted in late and very late resorptions.
No effect is observed in rats by the dermal route up to 1000 mg/kg/d (Hellwig 1993).
An increase in post-implantation loss was also observed at the highest dose by the oral route in rabbit in presence of maternal toxicity (Hellwig 1990a) and consisted mainly of early loss in contrast to rats.

In the two-generation study (Hellwig 1992) a significant decrease in mean litter size is seen at the highest dose in F1a and F1b that may be consistent with an effect on post-implantation loss.

Altogether, these data indicate that the induction of post-implantation loss by epoxiconazole is worsened with the extension of the duration of exposure at the end of gestation with higher rate of resorptions and later stages of resorptions observed. Post-implantation loss was observed in prenatal developmental toxicity studies, in which dams were sacrificied before parturition. It is considered that dystocia may not have contribute to the induction of resorptions. Induction of post-implantation loss was observed in the Taxvig studies in absence of significant maternal toxicity. Therefore, it cannot be considered secondary to non specific maternal toxic effects. In these studies, maternal toxicity was assessed by measurement of maternal body weight gain and clinical signs but it should be noted that maternal food consumption was not measured.

The hypothesis that this effect could be secondary to endocrine disruptive effects in the mother has been raised. However, no correlation between the progesterone level in dam plasma and the rate of very late resorptions was identified from an analysis of individual data from the Taxvig 2007 and Taxvig 2008 studies. It should however be noted that available data on hormonal effects of epoxiconazole in dams show a consistent significant effect on oestradiol and testosterone levels but not on progesterone. In Schneider 2002 both oestradiol reductions and induction of late resorptions were observed. Besides, another aromatase inhibitor – letrozole - has effects on maternal levels of oestradiol but not of progesterone in monkeys (Albrecht 2000). In rats, letrozole also induces an increase in late resorptions that is prevented by co-exposure to estrogen (Tiboni 2009). This tends to demonstrate that late resorptions in rats may be linked to endocrine disruptive effect of aromatase inhibitors in the dams via oestradiol. It can be argued that due to differences in hormonal regulation of gestation between species, a doubt on human relevance could be raised for such a mechanism of action. However, in absence of clear data to establish the mechanism of action of epoxiconazole for induction of late resorptions, **no conclusion can be made on the potential absence of relevance for humans**.

Teratogenic effets

Several prenatal developmental toxicity studies are available and provide information on induction of malformations and variations. The two-generation study is also relevant for external malformation (Hellwig 1992). Main data on malformations, variations and level of general maternal toxicity such as effect on maternal body weight or food consumption are summarised in Table 49 below.

Table 49 Summary of information on malformations and variations.

Hellwig 1989	Dose (mg/kg/d)	0	20	60	180
OCDE 414 range-finding Oral Wistar rat (n=25) GD6-15 (only external exam.)	Maternal corrected bw gain	-	**-16%***	**-24%***	**-49%***
	Maternal food conso GD6-15	-	-6%	-13%	**-27%***
	% foetus with malformation	0%	0.7%	0%	**50.2% ***
	% foetus with cleft palate	0%	0.4%	0%	**50.2% ***
	% foetus with anasarca	0%	0%	0%	0%
Hellwig 1990b	Dose (mg/kg/d)	0	5	15	45

OCDE 414 Oral Wistar rat (n=25) GD6-15	Maternal corrected bw gain	-	-1%	+1%	-1% (transient bwg ¬ GD6-8)
	Maternal food conso GD6-15	-	-2%	-2%	-7%
	Maternal food conso GD6-8	-	-1%	-1%	**-13%***
	Maternal food conso GD13-15	-	-2%	-3%	**-7%***
	% foetus with malformation	0.7%	1.1%	1.5%	2.0%
	% foetus with cleft palate	0%	0%	0.3%)	0%
	% anasarca	0	0	0	0
	% foetus with variations	37.1%	33.0%	43.7%	**52.9%***
	% foetus with rud. cerv. ribs	6.3%	1.1%	7.6%	**15.9%***
	% foetus with access. 14th rib	0	1.1%	**4.1%***	**24.8%***
Schneider 2002	Dose (mg/kg/d)	0	180 (batch 1)	180 (batch 2)	
OCDE 414 with a single dose Oral Wistar rat (n=25) GD6-19	Maternal corrected bw gain	-	**-45%***	**-30%***	
	Maternal food conso GD6-19	-	-14%	-6%	
	Maternal food conso GD6-8	-	**-30%***	-6%	
	Maternal food conso GD15-20	-	**-20%**	**-17%***	
	% foetus with malformation	1.2%	**42%**	5.9%	
	Foetus with cleft palate (%)	0	2 (2.4%)	1 (0.8%)	
	Foetus with anasarca (%)	0	5 (6%)	2 (1.7%)	
	% foetus with sk. variations	98.9%	100%	100%	
	% foetus with cervical ribs	1.2%	40%	15%	
	% foetus with supern. 14th rib	4.7%	36%	32%	
Hellwig 1993	Dose (mg/kg/d)	0	100	400	1000
OCDE 414 **Dermal** Wistar rat (n=25) GD6-15	Maternal corrected bw gain	No effect reported			
	Maternal food consumption	No effect reported			
	% foetus with malformation	Not reported			
	% foetus with cleft palate	0	0	0	0.3%
	% foetus with anasarca	0	0	0	0
	% foetus with variations	Not reported			
	% rud. cervical ribs	≈ 0.6%	≈ 0.6%	≈ 2.5%	≈ 3.5%
	% 14th ribs	≈ 0.3%	≈ 0.3%	≈ 1.9%	≈ 2.9%
Hellwig 1990a	Dose (mg/kg/d)	0	5	20	80
OCDE 414 Oral **Rabbit** (n=15) GD7-19	Maternal corrected bw gain	-	-44%	-17%	-13%
	Maternal food conso GD7-19	-	**-16%***	**-13%***	**-13%***
	% foetus with malformations	4%	3.2%	0	0
	Foetus with cleft palate (%)	0	1 (1.1%)	0	0
	Foetus with anasarca (%)	0	0	0	0
	% foetus with variations	1.3%	1.1%	1.4%	3.6%
Hellwig 1992	Dose (mg/kg/d)	0	~ 0.9	~ 2.3	~ 23
OCDE 416 Oral Wistar Rat	Maternal F0 food conso	-	-	-	-13% in lactation of F1a

(n=25) 0-10-25-250 ppm	Anasarca F1a F1b	0 0	0 0	0 0	0 1 (0.4%)
	Cleft palate F1a F1b	0 0	0 0	0 0	0 1 (0.4%)
	Maternal F1 food conso	-	-	-	-6%* GD14-20 and -12% in lact.
	Anasarca F2	0	0	0	3 (3)
	Cleft palate F2	0	0	0	0

Several aspects related to the teratogenic potential of epoxiconazole were noted.

Epoxiconazole induces skeletal variations in the rat by oral route but not in the rabbit. In particular additional cervical and 14th ribs are observed. These variations are very commonly observed with other triazole compounds. For epoxiconazole, observation of a 14th rib occurs with a dose-response relationship and statistically significantly from the mid-dose without maternal toxicity in Hellwig 1990b. However, variations are considered of lower concern for classification and this effect is not sufficient to justify a classification Repr. Cat. 1B (CLP).

Two malformations are repeatedly identified.

Anasarca (generalised oedema) is a rare malformation and it was observed above historical control values in two studies in rat by oral route and not in rabbit. In Schneider 2002, they were observed in presence of maternal toxicity. In the two-generation study (Hellwig 1992), this malformation was present only in stillborn or pups that died early at high dose. At this dose level, prolonged duration of pregnancy was observed and it is not clear whether this malformation may be secondary to difficulty in parturition or to a teratogenic effect. Based on this uncertainty this effect is not sufficient to justify a classification Repr. Cat. 1B (CLP).

A very high rate of **cleft palates** (50% of foetuses, 90% of litters affected) was observed in the rat by oral route in Hellwig 1989 at the high dose of 180 mg/kg/d. Such an increase was not reproduced at the same high dose in Schneider 2002 in none of the two purity batch, with cleft palates observed in only 2 (2.4%) and 1 (0.8%) foetuses. However, in this study, the high rate of post-implantation loss (respectively 59 and 43%) may have masked teratogenic effects. Maternal toxicity was noted at this dose level in both studies as evidenced by decreases in food consumption and significant decrease in corrected maternal body weight gain (-45 and -30%). One cleft palate was also observed at the low dose (20 mg/kg/d) in Hellwig 1989.

In the other prenatal developmental toxicity studies, one cleft palate was also identified at the mid-dose (15 mg/kg/d) in rat by oral route in Hellwig 1990b, one at the high dose (1000 mg/kg/d) in rat by dermal route (Hellwig 1993). Besides, one cleft palate was reported in the two-generation study (Hellwig 1992) at the highest dose in F1b (approx. 23 mg/kg/d). No maternal toxicity was observed at these dose levels in these rat studies.
In the rabbit, one cleft palate was observed at the low dose (5 mg/kg/d) by oral route (Hellwig, 1990a). However, in the absence of such findings at the mid- and high-doses, its significance is unclear.

Cleft palate is a rare malformation with available historical control data in rats showing that 1 foetus with a cleft palate may be spontaneously observed on rare occasions (historical control mean: 0.06%; range: 0-0.2.% in Hellwig 1990b indicating twice 1 cleft palate observed in 10 studies). Occurrence of one cleft palate in one study is therefore consistent with historical controls and

cannot be unequivocally attributed to treatment. However, the repetition of this isolated finding in all five rat prenatal developmental toxicity studies that investigate malformations supports the conclusion that they are not of spontaneous origin and that they are biologically significant.

The absence of a dose-response in two of the studies (Hellwig 1989 and Hellwig 1990b) also raises an uncertainty on the relation of this malformation with treatment. However, considering the general low occurrence of this finding, a very large number of animals would be necessary to expect a clear dose-response and the biological significance should be given greater importance.

Besides, cleft palate is a malformation that is commonly observed with triazoles compounds in the presence or in the absence of maternal toxicity. It is a very specific malformation implying a disturbance in the process of craniofacial morphogenesis and several modes of action have been proposed. Menegola 2006 suggest that triazoles may inhibit the embryonic CYP450 (CYP26) involved in the regulation of retinoic acid, whereas an alternative hypothesis involving blockade of IKr potassium channel, embryonic arrhythmia and hypoxia has also been proposed, based on data for ketoconazole (Ridley 2006, Danielsson 2007). However, none of these modes of action have been studied for epoxiconazole.

Overall, RAC considers that based on a weight of evidence approach and considering the specificity and the spontaneous infrequency of this malformation otherwise commonly seen with triazoles, the induction of a high incidence of cleft palates in the presence of maternal toxicity (Hellwig 1989) and the repeated observation of isolated cleft palates in rats at doses without maternal toxicity enable **a clear identification of cleft palate as a developmental effect** of epoxiconazole. It is considered that induction of cleft palates cannot be attributed to maternal toxicity such as decreased food consumption or reduced body weight gain and **it cannot be considered secondary to other maternal toxic effects**.

Peri- and postnatal deaths

Two post-natal studies are available and provide relevant information on this aspect, i.e. the two-generation study and the post-natal part of Taxvig 2007.

Main data on peri- and post-natal deaths and level of general maternal toxicity such as effect on maternal body weight or food consumption are summarised in Table 50 below.

Table 50 Summary of information on peri- and post-natal deaths.

Taxvig 2007	Dose (mg/kg/d)	0	15	50	
Oral Wistar rat (n=6,9,18) GD7-GD21	Maternal toxicity : bw gain GD7-PND1	-	-12%	-27%	
	Maternal food consumption	Not measured			
	Maternal dystocia	0	0	4/7	
	Maternal gestation length (d)	22.46	22.67	**23.71***	
	% perinatal loss	9.67%	18.21%	**88.79%***	
	% post-natal deaths	3.39%	2.78%	**69.44%***	
Hellwig 1992	Dose (mg/kg/d)	0	~ 0.9	~ 2.3	~ 23
OCDE 416 Oral Wistar Rat	Maternal F0 food conso	-	-	-	-13% in lactation of F1a

(n=25) 0-10-25-250 ppm	Maternal toxicity F0: vaginal hemorrhages	-	-	-	6 dams (F1a)
	Prolonged duration of pregnancy F1a F1b	0 3	0 1	2 4	**9*** 3
	Mean litter size F1a F1b	13.3 13.8	12.3 13.7	13.7 13.0	**9.1*** **12.2***
	Viability index F1a F1b	90 95	90 94	92 93	93 **90***
	Lactation index F1a F1b	99 100	99 99	99 99	99 99
	Maternal F1 food conso	-	-	-	**-6%*** GD14-20 and -12% in lact.
	Maternal toxicity F1: vaginal hemorrhages	-	-	-	1 dam
	Prolonged duration pregnancy	2	0	2	**6***
	Mean litter size F2	11.7	12.9	10.8	10.9
	Viability index F2	98	**93***	**94***	**82***
	Lactation index F2	99	98	98	**94***

Perinatal deaths are defined in the Taxvig study as the number of live pups at weaning relative to the number of implantation. They therefore account for post-implantation loss as well as postnatal deaths. The observation of perinatal deaths is therefore consistent with the level of post-implantation loss identified in the caesarean part of the same study.
This effect may also explain the slight but significant decrease in mean litter size seen at the highest dose in the two-generation study in F1a and F1b. At this dose level, a longer duration of gestation is observed in several dams and difficulty in parturition may also in part explain this effect as dystocia is commonly associated with hypoxia, foetal distress and mortality just before and after birth.

An statistically significant increase in post-natal deaths is also observed at the high dose in Taxvig 2007. At this dose a large number of dams experienced dystocia and it is therefore not possible to exclude that the post-natal deaths are secondary to maternal dystocia. In the same way, viability and lactation index were slightly but significantly decreased below historical controls value in F2 at the highest dose. However, prolonged parturition is also observed at this dose level and may have contributed to the observed effect.

Based on the uncertainty associated with the role of dystocia in difficulties in parturition on post-natal deaths, this effect is not sufficient to justify a classification Repr. Cat. 1B (CLP).

Additional information on the quality of the studies

It should be noted that several weaknesses were noted regarding Taxvig studies that are a key element in the conclusion related to post-implantation losses.
The Taxvig studies were designed specifically to investigate the developmental endocrine potential of epoxiconazole and were not performed according to guidelines and GLP. In particular, the studies included a limited number of animals (6 to 14 instead of 20 in guideline studies) but the resulting reduction of the power of the study is not considered as a concern as an effect is identified. The increase in post-implantation loss was repeatedly observed in both Taxvig studies and the effect was considered as scientifically robust and clear. Besides, maternal toxicity was assessed by measurement of maternal body weight gain and clinical signs but it should be noted that maternal

food consumption was not measured. Finally, the very low level of post-implantation loss in control animals –none reported whereas historical controls from other rat studies always exceed 3.8%- was also pointed out in Taxvig 2008 but control values in Taxvig 2007 were in the usual range.
The other prenatal developmental toxicity and two-generation studies were performed according to GLP and OECD guidelines. Deviations were noted in Schneider 2002, in which only one dose was tested but with two different batches of different purity and in Hellwig 1989, in which visceral and skeletal examination of the foetuses was not performed. As full external examination of the foetuses was performed, the latter study was considered to provide scientifically valid data to evaluate external malformations such as cleft palates.

Overall conclusion

The level of evidence for induction of post-implantation loss and cleft palates is in agreement with the criteria for CLP classification Repr. Cat. 1B that "available data provide **clear** evidence of an adverse effect [...] on development in the absence of other toxic effects or if occurring together with other toxic effects the adverse effect on reproduction is considered **not to be a secondary non-specific consequence of other toxic effects**". Besides, in the absence of relevant mechanistic information **it cannot be concluded "that there is a doubt about the relevance of the effect for humans**" implying that "classification in category 2 may be more appropriate".

The induction of post-implantation loss and cleft palates by epoxiconazole therefore justifies a developmental classification in Cat. 1B (CLP).

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. Epoxiconazole has a harmonized classification for developmental toxicity in Annex VI of CLP Regulation and revision of its classification for developmental toxicity as proposed in this dossier need to be discussed on a community-wide basis in accordance with article 36 of CLP.

OTHER INFORMATION

For all references, see REFERENCES.

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APPENDIX I – Communication of Ulla Hass circulated to the RAC futher to RAC-9.

D. 9/2 2010
Ulla Hass, FOOD-DTU

Further information in relation to the Taxvig et al 2007 and 2008 studies performed in our laboratory

Maternal toxicity
Note that the GD 21 dam body weight in Taxvig et al 2008 is the **adjusted** dam body weight, i.e. dam body weight minus uterus weight. The mean values and std. (in g) are shown in the table below. There are no statistically significant differences.

Group	N	Dam bw, GD 21	Uterus weight, GD 21	Adjusted dam body weight, GD 21
Control	6	286,17	62,18	223,98
Epoxy - 50	8	297,38	79,19	218,19
Std.				
Control		27,52	13,07	20,39
Epoxy - 50		14,35	11,72	10,93

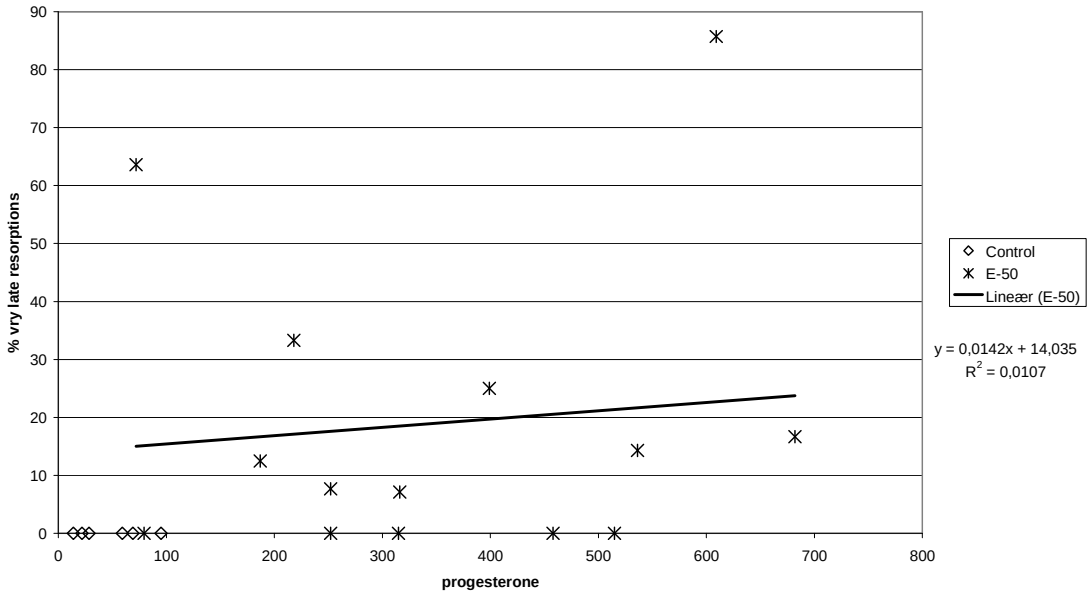
Progesterone versus very late resorptions
The % very late resorptions in litters versus the dam progesterone level in plasma on GD 21 in Taxvig et al 2007 and 2008 is shown on the next page.

In Taxvig et al 2007, there is no correlation between the two endpoints ($R^2 = 0.01$).

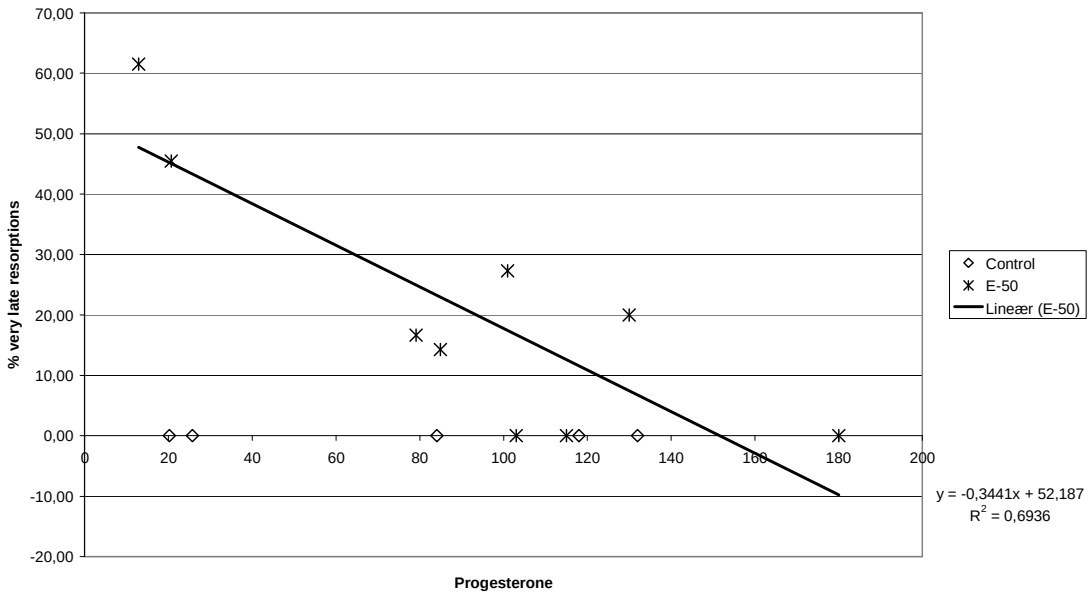
The results in Taxvig et al 2008 seem to indicate a negative correlation ($R^2 = 0.69$), but this result may be coincidental, because the variation in the dam progesterone levels are smaller in this study than in the Taxvig et al 2007 study.

Overall, the results do not support a link between high progesterone in dams and high % very late resorptions.

Study 05-16 % very late resorptions vs. progesterone in dam plasma GD 21(Taxvig et al 2007)



Study 06-15 % very lat resorptions vs. progesterone in dam plasma GD 21 (Taxvig et al 2008)



Definition of resorptions and historical control for late resorptions and very late resorptions, i.e. dead foetuses

The definitions in the OECD TG 414 Prenatal developmental toxicity study was used, i.e.:
Resorptions: a conceptus which, having implanted in the uterus, subsequently died and is being, or has been resorbed
Early resorption: evidence of implantation without recognisable embryo/foetus
Late resorption: dead embryo or foetus with external degenerative changes

During the sectioning of the dams, we observed several very late resorbtions/dead foetuses and used the following definition:
Very late resorptions: dead foetus with small or no external degenerative changes, developed at least to GD 15.
In the OECD TG 414, the term for this appear to be dead foetus.

Control data for late resorptions and very late resorptions from some other studies on epoxiconazole as well as some recent historical control data from our lab is shown in the table below. This illustrates that late resorbtions may occur with a low frequency in control Wistar rats (0-6%), whereas very late resorptions/dead foetuses are very rare (0%) in control groups.

Historical control data for late resorptions and very late, i.e. dead fetuses in Wistar rats

Study	% late resorptions	% dead foetuses
Hellwig and Hildebrand 1989	~ 2% (0.3 of 14.7)	0%
Hellwig and Hildebrand 1990b	~0.7% (0.1 of 14.3)	0%
Schneider et al 2002	~5.6% (0.5 of 9.1)	0%
Hansen et al 2009, Tox. Sci. (nitrate study in our lab)	0% (5.3% early resorptions)	0%
Taxvig et al 2008, Tox. Sci (paraben study in our lab)	0% (10.6% early resorptions)	0%

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Definition of peri- and postnatal loss
% perinatal loss = (no. implants-no. live at weaning)/no. implants
% postnatal death/loss= (no. pups at birth-no. live at weaning)/no. pups at birth
% postimplantation loss= (no. implants-no. pups at birth)/no. implants (NB May in studies where the animals give birth include some live or dead offspring eaten by the mother before recording no. pups at birth)

In Taxvig et al 2007, the data for the 4 pregnant dams sacrificed on GD 24-25 due to dystochia in the E-50 group showed the following:
Dam 51, GD 25: 1 live foetus, 4 dead foetuses and **8 late resorptions**
Dam 53, GD 24: 0 live foetuses, 8 dead foetuses and **2 late resorptions**
Dam 54, GD 24: 8 live foetuses, **3 late resorptions and 1 very late resorbtion**
Dam 55, GD 23: 9 live foetuses and 1 early resorbtion
The late and very late resorptions in the 3 of the 4 dams were dead before GD 21, i.e. at least 2 days before any signs of dystochia.

Comment: Maternal toxicity in 2-gen study at highest dose of 250 ppm (21-31 mg/kg/day)?

It was stated during the RAC meeting, that there was maternal toxicity at the highest dose level in the two-generation study. However, the following information is given in the Annex XV dossier, December 8, 2008:

- There was no effect on body weight and body weight gain in Fo parental animals (only effect in F1 males).
- There was reduced food consumption in Fo females during lactation of F1a litters. The mean number of pups per dam is 9.1 compared to 13.3 pups/litter in the control group, i.e. ~30% fewer pups. The lower number of pups is a very likely explanation for the lower food consumption in the lactating animals and not a sign of maternal toxicity. Anyway, the significantly fewer pups born cannot be due to decreased food consumption **after** birth.
- There was no effect on clinical chemistry in Fo parental animals.
- There was no effect at pathology examination, in Fo parental animals, incl. no effect on liver weight (only seen in F1).

Consequently, my evaluation is: There are no signs of maternal toxicity in the Fo parental animals during pregnancy at highest dose of 250 ppm (21-31 mg/kg/day).