

**Committee for Risk Assessment**  
**RAC**

**Annex 4**  
**to the RAC Opinion on toxicity**  
**to reproduction of**  
**Epoxiconazole**

ECHA/RAC/A77-O-0000001412-86-08/A3

**Adopted**  
**28 November 2012**

## **Annex 4**

### **Summary of the Additional Information Report (AIR) and RAC comments**

The submitter of the AIR has generated new toxicological data in the process of clarifying the endocrine disruption potential of epoxiconazole according to requirements of the Annex I Inclusion Directive 2008/107/EC. BASF has been requested to submit all data available by 1 March 2012 to the EU for the purpose of data evaluation and consideration for appropriate classification and labelling of epoxiconazole by the Risk assessment Committee (RAC) of ECHA.

The new study data provides useful and relevant information. Robust summaries of these investigations are included in the AIR. A conclusion on the relevance of the new information for the development toxicity classification of epoxiconazole is also provided including a clarification of the added value of the new information in comparison to already considered information in the RAC opinion from March 2010.

#### **Toxicity for reproduction – Developmental toxicity data**

##### **Animal data**

BAS 480 F (epoxiconazole) - Modified prenatal developmental toxicity study in Wistar rats – oral administration (gavage) – S. Schneider et al, 2010 – Study number: 2010/1062087 (00R0307/00R001) and Amendment No. 1 to the report - BAS 480 F (epoxiconazole) – Modified prenatal developmental toxicity study in Wistar rats - Oral Administration (gavage) – S. Schneider and R. Moreno, 2011 – Study number: 2011/1229835 (00R0307/00R001)

Epoxiconazole was tested to obtain information on a reported<sup>1</sup> possible developmental effect of epoxiconazole, in particular foetolethality during late pregnancy. The study was designed to clarify the relationship of observed foetolethality to potential dysregulation of sex hormones and to investigate the impact of the vehicle used for test compound administration. The test material was administered orally by repeated gavage to female Wistar rats from gestation day (GD) 7 through GD 18 or 21. Maternal serum concentrations of progesterone, oestradiol, testosterone and androstenedione were determined on GD 18 or 21 to monitor gestation-stage dependent changes in hormone levels.

Epoxiconazole was administered to pregnant Wistar rats by gavage at doses of 23 and 50 mg/kg body weight, daily as a suspension in corn oil or as a suspension in 1% carboxymethylcellulose suspension in highly deionized water from GD 7 to GD 18 and from GD 7 to GD 21.

##### *Conclusions from the submitter of the AIR:*

When administered in corn oil or in carboxymethyl cellulose (CMC) from GD 7 to GD 18, epoxiconazole induced maternal toxicity at 50 and 23 mg/kg/day (decreased food consumption, decrease in carcass weight and corrected net weight gain, decreased platelet count, decreased oestradiol and progesterone values, increased androstenedione and testosterone values). Anemia and effects on proteins, glucose and calcium levels were observed only when corn oil was used. At 50 mg/kg/day, there was a clear increase in late resorptions and a decrease in the percentage of live fetuses.

When the same doses were given in the same vehicles from GD 7 to GD 21, the maternal toxicity was enhanced at both dose levels and especially with corn oil as

the vehicle (vaginal hemorrhages, piloerection, clear anemia). The percentages of late resorptions and post-implantation loss were also increased with a longer duration of treatment. Eventually, increased incidence of necrobiotic placentae was recorded at 50 mg/kg/day in CMC.

It was concluded that the results of this study are in agreement with Taxvig conclusions in 2007. However, BASF established a correlation between the maternal toxicity (in presence of hormonal changes) and the increase in late resorptions.

*RAC comments:*

RAC questions the conclusion of the submitter of the AIR that the increase in embryofetal death/resorption is a consequence of the maternal toxicity without establishing a clear relationship between hormonal changes and embryofetotoxicity.

In addition, the study does not explore the possible mechanism of induction and/or prevention of dysmorphogenesis (i.e., presence of cleft palate) seen with theazole class of compounds, at least in murid species.

BAS 480 F (epoxiconazole)- Modified prenatal developmental toxicity study in Wistar rats – oral administration (gavage) -Subcutaneous coadministration of Oestradiol cyclopentylpropionate – S. Schneider et al., 2010 – Study number: 2010/1062088(00R0307/00R002) and Amendment No. 1 to the report - BAS 480 F(epoxiconazole) – Modified prenatal developmental toxicity study in Wistar rats-oral administration(gavage) - Subcutaneous coadministration of Oestradiol cyclopentylpropionate – S. Schneider et al, 2011 – Study number: 2011/1229836(00R0307/00R002)

Epoxiconazole was tested to obtain information on a developmental effect of this test compound in rats, in particular embryo/fetoletality during late pregnancy. The study was designed to clarify the relationship of observed fetoletality to potential dysregulation of sex hormones. For this reason, pregnant rats were co-administered oestradiol cyclopentylpropionate (ECP) to test the capacity of estrogen supplementation in preventing epoxiconazole-mediated embryo/foetoletality. Epoxiconazole was administered orally by repeated gavage at a standard dose of 50 mg/kg body weight/day and oestradiol cyclopentylpropionate (ECP) was co-administered at doses of 0.5 or 1 µg/animal/day by subcutaneous injection to presumed pregnant female Wistar rats daily from gestation day (GD) 7 through GD 21. Maternal serum concentrations of progesterone, oestradiol, testosterone and androstenedione were determined on GD 21 to monitor gestation stage dependent changes in hormone levels and the influence of oestradiol supplementation.

Epoxiconazole was administered as a suspension in corn oil and oestradiol cyclopentylpropionate (ECP) was administered as a solution in corn oil.

*Conclusions from the submitter of the AIR:*

The dose of 50 mg/kg/day with a co-administration of ECP either at 1 or 0.5 µg/animal/day induced maternal toxicity (piloerection, vaginal hemorrhage, decreased food consumption and decreased net corrected body weight gain, anemia, decreased total protein, albumin and globulin values, increased phosphate, glucose, triglycerides and cholesterol). There was a decrease in the oestradiol values and an increase in androstenedione and progesterone values. There was no adverse consequence of these changes on fetuses.

In conclusion, there is a correlation between the oestradiol reduction and the fetal mortality. The supplementation of ECP during pregnancy prevents fetal mortality.

The histopathological examination of pregnant females' organs and placentae showed the expected effects of aromatase inhibition with azole compounds on the liver and adrenals. The placentae were increased in size, volume and weight and presented a degeneration of the labyrinth and trophospongium. These effects were not dose related and independent of the vehicle used. Placentae of the late resorptions were in general more affected than those of live foetuses.

*RAC comments:*

This study shows the correlation between the oestradiol level and the maintenance of pregnancy in rats and the role of aromatase inhibitors in the detrimental effects on the hormonal cascade. However, the study does not explore the possible mechanism of induction and/or prevention of dysmorphogenesis (i.e., presence of cleft palate) seen with the azole class of compounds, at least in murid species.

BAS 480 F (epoxiconazole) - Modified maternal toxicity study in guinea pigs Oral administration (gavage) - S. Schneider et al, 2011 - Study number: 2011/1229837 (Project No.52R0307/00R003)

Epoxiconazole was tested in this range-finding study in pregnant Dunkin-Hartley guinea pigs to determine a dose which produces either some effects on maternal toxicity or toxicity in the offspring, to facilitate the selection of the dose levels for a subsequent definitive prenatal developmental toxicity study.

The test material was administered by gavage as a suspension in 1% carboxymethylcellulose suspension in highly deionized water (1% CMC) at doses of 5, 15, 50 and 180 mg/kg body weight/day on gestation day (GD) 6 through GD 63

Maternal serum concentrations of a variety of steroid hormones were determined twice during the study to monitor gestation-stage dependent changes in hormone levels.

At terminal sacrifice on GD 63 fifteen to seventeen females per group had implantation sites.

All animals from test group 4 (180 mg/kg bw/d) were sacrificed prematurely. At the time of sacrifice, the dose of 180 mg/kg was lethal for 7 out of 20 animals in this group and caused further distinct signs of clinical toxicity in the remaining animals, such as abortion, poor general state, as well as distinctly reduced food consumption and loss of body weight. The exaggerated toxicity in these animals prevented a meaningful assessment of potential developmental effects. Thus, all data gathered until interim sacrifice of this test group are not reported.

*Conclusions from the submitter of the AIR:*

The dose of 180 mg/kg bw/d killed one third of the animals until mid-pregnancy and caused distinct signs of intoxication in the surviving animals of this group.

At the other dose levels, 5, 15 and 50 mg/kg bw/d, neither significant signs of systemic toxicity nor effects on reproduction or offspring were detected.

The only remarkable observations were alterations in steroid hormones starting at a dose of 5 mg/kg bw/d. The increase of corticoids, mainly at 50 mg/kg bw/d, suggests a higher steroid hormone production of the adrenals, possibly related to stress.

Based on these conclusions, doses of 15, 50 and 90 mg/kg bw/day were chosen for the definitive modified prenatal developmental toxicity study.

*RAC comments:*

It should be noted that the dose of 180 mg/kg/day which was also used in rat studies induces a greater toxicity in pregnant guinea pigs, probably related with a greater internal exposure (see toxicokinetic data below). It should also be noticed that from the lowest dose level the hormonal balance (corticoids and steroids) was altered.

BAS 480 F (epoxiconazole) - Modified prenatal developmental toxicity study in guinea pigs - Oral administration (gavage) - S. Schneider et al, 2011 - 2011/1229838 (Project No.54R0307/00R005)

Epoxiconazole was tested for its prenatal developmental toxicity in Dunkin Hartley guinea pigs. The test substance was administered as a suspension in 1% carboxymethylcellulose suspension in highly deionized water (1% CMC) at doses of 15, 50 and 90 mg/kg body weight on gestation day (GD) 6 through GD 63.

Maternal serum concentrations of a variety of steroid hormones were determined to monitor gestation-stage dependent changes in hormone levels. All placentas and ovaries were examined histopathologically.

*Conclusions from the submitter of the AIR:*

The dose of 90 mg/kg bw/d caused a mild anemia and signs for a higher steroid hormone production of the adrenals, possibly related to stress in pregnant guinea pigs. It is noteworthy, that other than in rats, histopathology of all placentas revealed no adverse effect caused by epoxiconazole up to and including a dose of 90 mg/kg/bw/d. Thus, the no observed adverse effect level (NOAEL) for maternal toxicity was set at 50 mg/kg body weight/day.

Steroid hormone level alterations were observed starting at a dose of 15 mg/kg bw/d. The increases of oestradiol precursors such as testosterone and androstenedione may suggest that guinea pigs are sensitive to aromatase inhibition. It is noteworthy, that other than in rats, histopathology of all placentas revealed no adverse effect caused by epoxiconazole up to and including a dose of 90 mg/kg bw/d.

Test substance-related, specific adverse effects on foetal morphology were not observed in this study, at the tested dose levels up to 90 mg/kg bw/d.

*RAC comments:*

This study clearly shows that guinea pigs are sensitive to aromatase inhibition and that the steroid hormones cascade is altered as in other test species such as rats. The absence of placental effects as seen in rats is likely due to the species differences in morphology and physiology of this organ. The absence of dysmorphogenesis might be, at least in part, due to a difference in the level of exposure (kinetic profile, Cmax and AUC) when compared to the rat.

BAS 480 F (epoxiconazole) - Pre-postnatal reproductive toxicity study in Guinea pigs - Oral administration (gavage) - S. Schneider et al, 2011 - Study number: 2011/1140627 (Project No.56R0307/00R011)

Epoxiconazole was tested in Dunkin Hartley guinea pigs for its potential effects on gestation, parturition, lactation and weaning, as well as on growth and development of the offspring. The test substance was administered as an aqueous formulation in 1% carboxymethylcellulose suspension in highly deionized water (1% CMC by gavage at doses of 15, 50 and 90 mg/kg body weight from gestation day (GD) 6 to end of gestation (about GD 65) and continued through weaning (postnatal day [PND] 21) until one day before necropsy.

Especially, the study provided information about the effects on duration of gestation and neonatal morbidity.

*Conclusions from the submitter of the AIR:*

The mid and high dose of 50 and 90 mg/kg bw/d caused signs for a altered steroid hormone production of the adrenals, which was at least at the high-dose level related to stress and maternal toxicity in pregnant guinea pigs. Thus, the no observed adverse effect level (NOAEL) for maternal toxicity is 15 mg/kg body weight/day.

No effects were noted on gestation, parturition and up-bringing of offspring, at the tested dose levels up to 90 mg/kg bw/d.

Test substance-related, specific adverse effects on pre- and postnatal development of offspring were not observed in this study, at the tested dose levels up to 90 mg/kg bw/d.

*RAC comments:*

Again, as in the above mentioned studies, this study demonstrates the guinea pig sensitivity to the aromatase inhibition and the perturbation of the steroids cascade induced by epoxiconazole.

BAS 480 F (epoxiconazole) - Prenatal developmental toxicity study in Wistar rats - Oral administration (gavage) - Subcutaneous co-administration of oestradiol cyclopentylpropionate - S. Schneider et al, 2011 - Study number: 2011/1229839 (Project No.00R0307/00R006)

Epoxiconazole was tested to obtain information on a developmental effect of this test compound in rats, in particular craniofacial malformations, on its relationship to aromatase-related oestradiol dysregulation. Oestradiol cyclopentylpropionate was coadministered to test the capacity of estrogen supplementation in preventing epoxiconazole mediated developmental effects. The results were generated to clarify the potential for endocrine disruption of the test compound, in accordance with requirements of Commission Directive 2008/107/EC (25 Nov 2008)<sup>1</sup> for further testing.

Epoxiconazole was administered orally by repeated gavage and oestradiol cyclopentylpropionate subcutaneously to female Wistar rats from GD 6 through GD 15. Epoxiconazole was administered (by oral gavage) at a standard dose of 180 mg/kg body weight/day to three groups of female Wistar rats, on gestation day (GD) 6 through 15. Two of these 3 groups were co-administered either 1 or 2 µg/animal/day oestradiol cyclopentylpropionate (ECP), which was injected subcutaneously.

*Conclusions from the submitter of the AIR:*

As known from previous studies, epoxiconazole adversely affected prenatal development of Wistar rats at 180 mg/kg bw/d, a dose which induced clear maternal toxicity in the form of reduced food consumption, body weight gain and oestradiol depletion. Salient adverse developmental effects were fetal deaths as well as craniofacial and tuberositas deltoidea malformations. Supplementation of oestradiol (1 or 2 µg/animal/d oestradiol cyclopentylpropionate) resulted in a modest dose-related increase of serum oestradiol levels, relative to the animals which received 180 mg/kg bw without supplementation. Nevertheless this modest increase of oestradiol was sufficient to prevent fetal deaths. With serum oestradiol levels still 88% below control levels supplementation with 2 µg/animal/d oestradiol was not effective against craniofacial and tuberositas deltoidea abnormalities.

*RAC comments:*

The co-administration of oestradiol confirms that the embryofetal deaths are related to the depletion of oestradiol necessary for maintenance of pregnancy in rats. However, dysmorphogenetic effects (including cleft palates) were seen at 180 mg/kg/day despite the oestradiol co-administration.

### **Human data**

Cases of fluconazole embryopathy have been reported (Pursley et al., 1996; Aleck et al., 1997; Lee et al., 1992; Carey et al., 2009; Lopez-Rangel and Van Allen, 2005). The anomalies reported included craniosynostosis, orbital hypoplasia, skeletal manifestation of humeral radial synostosis and femoral bowing, cleft palate, cardiovascular malformations and joint contractures. However, the actual teratogenic risk of high fluconazole exposure is difficult to estimate due to the absence of large epidemiological studies to estimate such a risk.

### **Other relevant information in in vitro studies**

BAS 480 F (epoxiconazole techn): Effect on HERG tail currents recorded from stably transfected HEK 293 cells – S. Hebeisen, 2010 - Study number:2010/1155854 (Project No.99V0307/00X001)

#### *RAC comments:*

This study has been designed to verify whether epoxiconazole may induce a QT-prolongation using an in-vitro model of HEK-293 cells exposed to epoxiconazole and in comparison with ketoconazole. Recent studies indicate that arrhythmogenic compounds induce dysrhythmia in foetal heart in all species and at lower dose levels than in adults (Danielsson, 2012). Therefore, positive results in a classical hERG study would be a good predictor of cardiac disturbances in embryo/fetal life and of known adverse consequences (cardiac, limb and/or orofacial malformations induced by hypoxia). In the present study, epoxiconazole blocked the hERG outward tail currents at any concentration above 1,0  $\mu$ M. This no effect dose in the hERG study is far below the internal exposure at the dose of 180 mg/kg which induced cleft palates in rat, i.e 28  $\mu$ M.

Such results do not dismiss the concern regarding the potential teratogenic effect of epoxiconazole and in addition, a potential adverse effect on development in juvenile animals may be indicated.

BAS 480 F (epoxiconazole) - Morphological and Immunohistochemical investigations in rat embryos cultured in vitro – E. Menegola, 2012 – Study number:2012/1058203 (Project No.00R0307/00X017)

The aim of the study was to generate information on the mode of action of epoxiconazole for induction of craniofacial malformations in rats as observed in vivo.

A number of azole derivatives are able to affect rat embryo development in vitro by altering the migration and distribution of cephalic neural crest cells into branchial arches (the embryonic precursors of facial elements).

In this study, the potential effect of epoxiconazole on development of branchial arches and possible induction of craniofacial abnormalities (cleft palate) was investigated in rat embryos of GD 11.5 after exposure to 0, 3, 10, 30, 60 and 91  $\mu$ M epoxiconazole during a critical time window of craniofacial development of 48 hours in a Whole Embryo Culture.

Additionally, the distribution of neural crest cells was assessed by immunostaining.

*Conclusions from the submitter of the AIR:*

The number of embryos with anomalies was significantly increased at and above 10 µM. The most common effect was fusion of branchial arches I and II and an anomalous branchial arch II. Also, an increased number of embryos with delayed or severely delayed otic vesicles were noted. From 30 µM fusion of branchial arches II and III occurred and above 60 µM single cases of embryos with fused somites or single pluri-malformed embryos were found.

No generalised effects on embryonic development were revealed in the morphometric evaluations.

The immunostaining showed an abnormal neural crest cell migration at the level of the branchial apparatus in embryos exposed to 30 µM and above.

BAS 480 F (epoxiconazole) Morphological and Immunohistochemical investigations in rat embryos gathered by C-section Oral Administration (Gavage) – B. Flick, 2012 – Study number: 2012/1059618 (Project No.00R0307/00R020)

The aim of this study was to gain further insight in the mode of action of epoxiconazole for induction of craniofacial malformations in rats as manifested in vivo during a critical period of prenatal craniofacial development. Female pregnant Wistar rats were exposed by repeated oral administration (gavage) at doses of 50, 100 or 180 mg/kg/day from gestational day (GD) 6 through 11. On GD 11 the explanted embryos were examined using morphological and immunohistochemical methods. The immunostaining of the whole embryos was performed in order to visualize neural crest cell (NCCs) migration and distribution.

The immunostaining of the samples was processed using antibodies against cellular retinoic acid binding protein (anti-CRABP) according to Menegola et al. (2003).

The embryos of the remaining dams of the high dose and control groups were prepared for the morphological investigation using the modified morphological score system (Klug S, 1985; Flick B et al., 2009).

*Conclusions from the submitter of the AIR:*

The compound caused substance related, adverse effects/findings at 180 mg/kg bw/d and to a minor degree at 100 mg/kg bw/d. Decreased food consumption, transient body weight loss or decreased body weight change were indicative of marked maternal toxicity at 180 mg/kg bw/d. At 100 mg/kg bw/d, marginal effects on body weight development were detectable on GD 6-8.

On GD 11, rat embryos were explanted from the treated pregnant rats and subjected to immunohistochemical investigations. These examinations were performed to clarify whether the craniofacial malformations in rat fetuses seen in previous studies after high-dose treatment with 180 mg/kg bw/d are preceded by dysmorphogenesis of the branchial apparatus; such a pathogenic pathway has been hypothesized to be involved in the induction of craniofacial malformations by triazoles (Menegola et al., 2006). No substance-related findings were observed in the morphology of the embryos at 180 mg/kg bw/d. The development of the exposed embryos was comparable to control embryos with regard to all endpoints of growth (grown-rump-length) and differentiation (number of somites and total morphological score – summarizing the scored development of different organ anlagen). Furthermore, no significant increase of dysmorphogenesis was observed in comparison to control. In contrast to a corresponding in vitro study investigating rat embryos of GD 11.5 after an in vitro exposure to epoxiconazole (Menegola et al, 2012) abnormalities and delays in development of branchial arches, optic and optical vesicles, somites and the neural tube were not observed under in vivo conditions in this study.



Under the study conditions, neural crest cell (NCCs) migration and distribution could only be visualized in 17 of the 37 investigated ex vivo GD 11 embryos. Therefore test substance related effects could neither be assessed nor be excluded. Further optimizations of the protocol are ongoing.

*RAC comments:*

As recognised by BASF, the data are not robust enough to draw a clear conclusion regarding the potential effect of epoxiconazole on the development of branchial arches and possible induction of craniofacial abnormalities (cleft palate). The effects of epoxiconazole and of its metabolites were not, therefore, fully evaluated in this Whole Embryo Culture (WEC) system. However it should be noticed that such dimorphogenetic effects have been seen when rat embryos are directly exposed to epoxiconazole in WEC system (Menegola et al, 2012).

**Toxicokinetics**

14C-BAS 480 F (epoxiconazole) – Kinetic study in pregnant Wistar rats – Fabian E., Landsiedel R., 2011 – Study number: 2011/1112619 (02B0277/03B001)

The aim of the study was to determine the kinetic profile of epoxiconazole in pregnant Wistar rats when administered orally at doses of 5, 50, 100 or 180 mg/kg/day of unlabelled test item from GD 6 to GD 9 and then with a single dose of the labelled compound on GD 10. Two vehicles were used: either aqueous carboxymethyl cellulose (CMC) or corn oil.

*Conclusions from the submitter of the AIR:*

The AUC values indicate an internal exposure (plasma exposure) consistent with the oral doses administered. The kinetic profile can be considered linear. At Tmax the range of mean plasma concentrations were 0.9 µg Eq/g at the lowest dose tested (5 mg/kg bw/d) and increased up to 20.18 µg Eq/g at the highest dose tested (180 mg/kg bw/d).

The use of corn oil led to a greater exposure to the test item (1.6 to 1.9 fold the AUC recorded with aqueous CMC)

*RAC comments:*

The kinetic profiles observed with each of the carrier seem consistent with the results of above mentioned studies where both vehicles were used.

Metabolism investigation of 14C-BAS 480 F (epoxiconazole) in plasma of pregnant Wistar rats after oral administration – Thiaener J. et al., 2011 – Study number: 2010/1031712(386207)

The objective of the study was to generate data on the metabolism and kinetics of epoxiconazole in pregnant Wistar rats. Female rats were dosed by the oral route for four days at 5, 50, 100 or 180 mg/kg/day with the unlabelled compound from GD6 to GD9 and then received a single administration of the labelled test item on GD10.

*Conclusions from the submitter of the AIR:*

Key finding on metabolism:

Metabolic transformation of epoxiconazole in Wistar rats led to the hydroxylated metabolite 480M02 and its isomer 480M26. Other metabolites present are 480M05 and its isomer, 480M60, 480M13 and its isomer and 480M06. Free 1,2,4 triazole (480M52) was mainly detected in the plasma samples from the last sampling occasions and constitutes the final degradation product of epoxiconazole.

*RAC comments:*

It should be noticed that the final degradation product of epoxiconazole in Wistar rats is the 1,2,4-triazole (480M52), known to be a potential teratogenic agent (INCHEM IPSC, reviewed April 2007<sup>1</sup>).

Kinetic study of 14C-BAS 480 F (epoxiconazole) in pregnant guinea pigs – Oral administration (gavage) – Thiaener J., Glaessgen W.E., 2011 – Study number: 2011/1108314(386904)

The biokinetics and biotransformation of epoxiconazole were investigated following oral administration (gavage) of 5, 15, 50 or 90 mg/kg of the compound from GD 6 to GD 9 and a radiolabelled corresponding dose of the compound on GD 10.

*Conclusions from the submitter of the AIR:*

Key finding on metabolism:

Metabolic transformation of epoxiconazole in guinea pig occurred mainly at two sites in the molecule leading to the formation of the triazole 480M02 or/and its isomer 480M26 and of a series of other metabolites (480M05, 480M60, 480M13, 480M54 and 480M52). Free 1,2,4-triazole (480M52) represented one of the two or three main metabolites in the plasma and thus a final degradation product of epoxiconazole.

*RAC comments:*

It should be noted that the final degradation product and one of the main metabolites of epoxiconazole in guinea pig is the 1,2,4-triazole (480M52) known to be a potential teratogenic agent (INCHEM IPSC, reviewed April 2007).

BAS 480 F (epoxiconazole) - Analysis in plasma of pregnant Wistar rats and in tissue of GD 11 embryos Oral Administration (Gavage) - Flick B. et al., 2012 – Study number: 2012/1058202 (Project No.00R0307/00R015)

The aim of this study was to determine epoxiconazole concentrations in plasma of pregnant Wistar rats and in tissue of embryos following repeated gavage administration of dams from gestational day (GD) 6 through 11.

Epoxiconazole was formulated in highly deionized water containing 1% carboxymethylcellulose suspension (1% CMC) and administered by gavage at doses of 5, 50, 100 and 180 mg/kg/day to groups mated female Wistar rats.

*Conclusions from the submitter of the AIR:*

The concentrations of epoxiconazole in maternal plasma sampled on GD 11 indicated an internal exposure that correlated with the oral dose levels. At Tmax the range of mean plasma concentrations were 0.48 mg/L at the lowest dose tested (5 mg/kg bw/d) and increased up to 9.2 mg/L at the highest dose tested (180 mg/kg bw/d). The concentrations of the test substance in embryonic tissue (representing yolk sac, amnion and the whole embryo) indicated a transplacental transfer of the compound. At the investigated time points the determined concentrations in the embryonic tissue were consistently lower than in maternal plasma. The concentration ratio between embryonic tissue and maternal plasma varied between 0.48 and 0.7 mg/mL at the different concentrations tested.

*RAC comments:*

It should be noted that the plasma levels of epoxiconazole at all dose levels, and especially at the highest dose of 180 mg/kg/day are high (order of the mg/mL). It would have been interesting to evaluate also the levels of the teratogenic 1,2,4-triazole metabolite in maternal plasma and foetal tissues.

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<sup>1</sup> 1,2,4-triazole – INCHEM IPSC - <http://www.inchem.org/documents/icsc/icsc/eics0682.htm>

### **Additional relevance of the provided information; choice of guinea pig**

Epoxiconazole (BAS 480F): Relevance of guinea pigs as model for developmental and reproductive toxicity testing - Stinchcombe S. et al., 2011 – Report number: 2011/1232616

*Conclusions from the submitter of the AIR:*

The guinea pig shares much more similarities with humans regarding pregnancy, gestational synthesis of estrogen and progesterone, intrauterine fetal development, and parturition when compared to rats. The species differences between murid rodents on the one hand, and guinea pigs and humans on the other, are most evident during the second half of gestation and initiation of parturition. This matches the time period in which adverse effects, namely late resorptions, prolonged gestation length, and dystocia occurred in rats but not in guinea pigs, although epoxiconazole was administered at maternally toxic dose levels to both species. In the light of the results obtained in recent studies with epoxiconazole and considering the remarkable species differences it is therefore very unlikely that the results in rats have predictive value for humans. Furthermore, the results of the new rat and guinea pig studies with epoxiconazole strengthen the evidence that the guinea pig is a model of first choice regarding effects on steroid hormone regulation during pregnancy with regard to humans.

With the guinea pig reproductive toxicity model it is possible to evaluate specific toxicological endpoints for which the rat is known not to be the appropriate/relevant model for extrapolation of the results to humans. Therefore, the guinea pig model is considered to be a very valuable and relevant complementary reproductive toxicity study model for special chemical classes (i.e. azoles) that are known to potentially influence steroid hormones levels involved in the regulation of pregnancy and parturition.

*RAC comments:*

This position paper consists of a good review of the recent literature regarding the use and the relevance of guinea pig as an animal model for reproductive and developmental studies. The position paper is divided in 4 parts, each comparing the guinea pig and murid rodents and bringing facts to show the relevance of this model for predicting the effects of xenobiotics in humans. Placentation, hormonal regulation of pregnancy and parturition, embryofetal development and reasons for the limited use of this model in reproduction toxicology, are reviewed.