Committee for Risk Assessment
RAC

Annex 5
to the RAC Opinion on toxicity to reproduction of
Epoxiconazole

ECHA/RAC/A77-O-0000001412-86-08/A5

Adopted

28 November 2012

COMMENTS AND RESPONSE TO COMMENTS ON A SPECIFIC ISSUE RELATED TO THE CLASSIFICATION AND LABELLING OF A SUBSTANCE FOLLOWING A REQUEST FROM ECHA'S EXECUTIVE DIRECTOR ACCORDING TO ARTICLE 77(3) OF REACH

ECHA has compiled the comments received via the internet as comprehensively as possible.

Substance name: Epoxiconazole
CAS number: 133855-98-8
EC number: 406-850-2
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
12/07/2012	Sweden		MSCA	1
Comment received				

Comments from the Swedish CA on the Public Consultation of the epoxiconazole AIR
We would like to thank IND for the very thorough presentation of the new toxicity data on epoxiconazole. Our comments contain 2 general comments on the new guinea pig studies, followed by detailed comments on the summary sections 3 and 4 ('Overall relevance' and 'Comparison with criteria'). We also include an appendix containing an expert review of the data by our consultant adviser Professor B. R. Danielsson.

General comments - Endocrine disruption
The aim of new studies performed by IND was to investigate the endocrine disrupting properties of epoxiconazole in compliance with Commission Directive 2008/107/EC. We note that the new guinea pig studies confirm that epoxiconazole affects the endocrine system, with the guinea pig being at least as sensitive as rats. Thus, in the end of the pregnancy, the hormone levels of estradiol (↓), progesterone (↓), and androstenedione (↑) are statistically affected in rats at 23 mg/kg/day (table 2/9 of the AIR), and the levels of androstenedione (↑), testosterone (↑), and deoxycortisol (↑) at 15 mg/kg/day in guinea pigs (table 2/41). The AIR also concludes that the guinea pig is sensitive to aromatase inhibition (page 93), and that the adrenal gland is the target organ of epoxiconazole in guinea pigs (page 114). In the pre-postnatal reproductive toxicity study in guinea pigs dose-dependent effects on adrenal hormones, weights, and pathology are evident at 50 mg/kg/day, and it should be discussed whether the adrenal toxicity meets the classification criteria for STOT RE. Thus, we conclude that the endocrine toxicity of epoxiconazole has been confirmed in an additional species.

General comments – guinea pig developmental toxicity
We would like to draw attention to the findings of 'thoracic centrum fused with arch' in the guinea pig developmental toxicity study. This effect occurs with a clear dose-response (litter incidences 26-52-63-72%), and with the effects in the two highest dose groups being statistically significant. Calculated as affected fetuses/litter, the percentage is 6.6-21.7-26.3-38.8%, with statistical significance in all treated groups. The AIR is surprisingly stating that the effects are either not dose-related or occurring only in the high-dose group, and that they are caused by delayed ossification caused by maternal toxicity/stress. It is not clear to us how the fusion of thoracic centrum and arch

can be caused by a delayed ossification. Furthermore, there are no signs of maternal toxicity (neither effects on food consumption or maternal body weight gain, nor effects on fetal weights). It seems correct that slight anemia and adrenal weight increases (defined as maternal toxicity in the AIR) were observed at the top dose, but increased incidences of 'thoracic centrum fused with arch' were observed already at the lowest dose level. We also note that the AIR defines this effect on the vertebrae as a variation, probably because 7 cases were observed also in the control group. However, as the flexibility of the vertebral column may be compromised by the fusion of centrum and arch, this effect is potentially adverse and borderline to a malformation. The AIR refers to a study by Rocca and Wehner (2009) as support for this effect being a common skeletal abnormality in guinea pigs. However, it should be noted that 'thoracic centrum fused with arch' was not observed in their study. Likewise, this effect is not observed in another study on guinea pigs by the same group (Wehner at al 2009). In our view, this study does not prove an absence of teratogenicity in guinea pigs, but rather indicates that also guinea pigs are sensitive to the developmental toxicity of epoxiconazole.

Comments on the summary section 3
Relevance of new information for developmental toxicity classification of epoxiconazole concerning the rat finding "post-implantation loss and resorptions"
The studies have shown that endocrine disruption, as evidenced by estrogen depletion caused by aromatase inhibition, is causing placental damage and late fetal death in rats. In view of the known differences in the hormonal regulation of late pregnancy between rats on the one hand and guinea pigs and humans on the other, we agree that the demonstrated 'placental' mechanism of action for induction of late fetal resorptions in rats may question the relevance to humans. However, as opposed to what is stated in the AIR, aromatase inhibition is a MoA that is highly relevant to humans, as evidenced by the therapeutic use of aromatase inhibitors (e.g. anastrozole) as anti-cancer drugs in the treatment of breast cancer.
Relevance of new information for developmental toxicity classification of epoxiconazole concerning the rat finding "malformations as cleft palate"
The MoA for the formation of cleft palates has not been identified, and the cleft palates therefore have to be assumed to be relevant for humans. As discussed by Menegola et al (2006), cleft palates are caused by many azoles and could therefore be considered as an azole class effect. There are many azole drugs used in medicine, and as discussed on previous RAC-meetings, some of them cause cleft palates in rats at high doses and 5 case reports discussed by Menegola et al (2006) indicate that fluconazole may cause malformations such as cleft palates also in humans. They are therefore having pregnancy labeling saying that they should not be used during pregnancy because of risk for malformations, showing that this MoA is considered to be of human relevance for other azole substances by the pharmaceutical authorities.
Concerning the MoA, effects on ion channels in the heart, and subsequent hypoxia, have been discussed in the AIR as a potential MoA. We do not agree with the interpretation of the HERG studies mentioned in the AIR, and our reasons are further outlined in the appendix. We still believe this is a plausible MoA, for which human relevance is well known.
New BASF rat developmental toxicity study data confirms that cleft palates induced at 180 mg/kg bw/d epoxiconazole occur in the presence of distinct maternal toxicity. The cleft palates were not prevented by estradiol co-treatment.
The AIR argues that maternal toxicity is the cause of the cleft palates. However, this explanation is not in line with the current scientific view on this issue. Thus, a recent review on the impact of

maternal toxicity on data interpretation concludes that conducted studies and literature data do not support that maternal toxicity (defined as clinical signs, decreased body weight gain or absolute body weight loss of up to 15 % in rats or 7 % in rabbits) can be used to explain the occurrence of major malformations (Beyer et al 2011). The review and the basis for this conclusion are further discussed in our appendix.

We find it proven by the performed studies that the estrogen hormone system (and the placental toxicity) is not involved in the generation of the cleft palates. We are therefore surprised to see that, in contrary what has been shown in the performed studies, the AIR suggests that the placental damage is causing the cleft palates. We can't see any scientific rationale for this statement.

New rat study data provides substantial evidence for a high-dose threshold for cleft palate formation, which is associated with marked maternal toxicity

We agree that a threshold for cleft palate induction seems to exist. However, such a threshold exists for most teratogens (basic principle in teratology-see text books). A threshold has also been shown for several other azoles which cause cleft palate as well as other malformations in rats only at high doses. Still, azoles used as drugs (including ketoconazole, itraconzole, fluconazole, voriconazole and posaconazole) have a similar pregnancy labeling despite that effects were only obtained at relatively high doses: "Due to developmental toxicity in animal studies the drug should not be used in pregnancy. Anticonception is recommended for women of childbearing potential". Furthermore, due to large species differences in disposition of compounds between humans and various animal species (e.g. metabolism, plasma protein binding, placental transfer, elimination pathways as well as intrinsic sensitivity of various tissues) it is not possible to establish safety margins based on mg/kg comparisons. For example, vitamin A derivatives (e.g. retinoic acid), which are highly teratogenic in humans (craniofacial malformations), cause similar teratogenicity in humans at 100 times lower doses (based on mg/kg) compared to doses which induce the same type of malformations in rodents. Cleft palates were not induced in guinea pig developmental toxicity studies when tested at up to 90 mg/kg bw/d (half the lethal dose in guinea pigs).

The absence of cleft palates in the guinea pig studies can not negate the findings of cleft palates in rats. Furthermore, the findings of dose-dependently increased incidences of vertebrae variations/malformation ('thoracic centrum fused with arch') indicate that also the development of guinea pigs can be adversely affected by epoxiconazole. Thus, the findings in guinea pigs may be considered supportive to the classification for developmental toxicity based on rat data.

BASF proposes that a rat-specific hormonal disruption, leading to a "rat-specific massive placental damage occurring during a critical period of organogenesis is a likely key event involved in the mechanism of action for epoxiconazole-mediated cleft palate formation", and that the cleft palates therefore are of limited human relevance. We are of the view that BASF has indeed proven that the MoA that they propose is incorrect, i.e., BASF has shown that estrogen supplementation blocks the placental damage without affecting the formation of cleft palates. Human relevance therefore has to be assumed for the cleft palates.

Comments on the summary section 4; Comparison with criteria

We agree that the mechanistic findings behind the post-implantation loss and resorptions in rats are reasons to doubt human relevance of these findings, and that these effects do not warrant classification.

Concerning the cleft palates, the signs of maternal toxicity that are mentioned in the AIR (anemia and hormonal disruption) do not provide explanations for the cleft palates. Furthermore, the proposed mechanisms are not supported by the available data. It is also claimed that aromatase

inhibition is of limited human relevance, but we have to reiterate that aromatase inhibitors are in fact used in human medicine, proving human relevance.

Overall, we do not think that the new data produced challenge the 1B classification for developmental toxicity based on the findings of cleft palates in rats. On the contrary, the findings of dose-dependently increased incidences of vertebrae variations/malformation ('thoracic centrum fused with arch') in guinea pigs further support developmental toxicity.

The effects noted on the guinea pig hormonal system give additional reason for concern. More specifically, dose-dependent effects on adrenal hormones, weights, and pathology are evident at 50 mg/kg/day in the pre-postnatal reproductive toxicity study, and it should be discussed whether the adrenal toxicity meets the classification criteria for STOT RE.

References

Beyer B., Chernoff N., Danielsson B.R. et al. (2011) ILSI/HESI Maternal Toxicity Workshop Summary: Maternal Toxicity and Its Impact on Study Design and Data Interpretation; Birth Defects Research (Part B) 92:36-51.

Menegola E et al (2006) Postulated pathogenic pathway in triazole fungicide induced dysmorphogenic effects. Reproductive toxicology 22; 186-195.

Rocca M S and Wehner N G (2009) The guinea pig as an animal model for developmental and reproductive toxicology studies. Birth Defects Research (Part B) 86:92-97.

Wehner N G et al (2009) Effects of natalizumab, an a4 integrin inhibitor, on the development of Hartley guinea pigs. Birth Defects Research (Part B) 86: 98-107.

*ECHA comment: The attachment document **Epoxiconazole_expert review Epoxiconazole: Evaluation of new reproductive toxicology studies by BASF and the potential regulatory impact of these new studies** [Epoxiconazole_expert review attachment to SE-CA comments.doc] was submitted as a separate attachment. See attachment no. 1 in appendix.*

RAC response

RAC fully agrees with Swedish CA comment on the quality of the work provided by BASF and the presentation of the various studies.

General comments on developmental toxicity

RAC fully agrees with the Swedish CA scientific justification and the conclusion that the study does not prove the absence of teratogenicity in guinea pig but rather indicates that also guinea pigs are sensitive to the developmental toxicity of epoxiconazole.

General comments on the summary section 3 – Overall relevance of the provided information

RAC supports this position.

Comparison on the summary section 4: comparison with the criteria

RAC supports the conclusion of the Swedish CA.

Date	Country	Organisation	Type of Organisation	Comment number
17/07/2012	Germany	BASF SE	Company-Manufacturer	2
Comment received				

p 217 - p 221, 4.1 Comparison with the CLP criteria:
"Based on a weight of evidence analysis, the classification of epoxiconazole in Category 2 for reproductive (developmental) toxicity under CLP is appropriate on the basis of findings of cleft palate in the rat. No classification is required for findings of post-implantation loss in the rat." (Overall conclusion of the expert opinion "Statement on the Classification of Epoxiconazole" prepared for BASF SE by Dr. David Andrew TSGE, UK, July 2012; the complete six-page scientific statement has been uploaded as comment to the additional information report)

*ECHA comment: The attachment document **Statement on the Classification of Epoxiconazole** [Statement on the Classification of Epoxiconazole_TSGE_July 2012.pdf] was submitted as a separate attachment. See attachment no. 2 in appendix.*	

RAC response
RAC disagrees with this position (for detailed justifications please see the opinion).

Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**
17/07/2012	Germany	BASF SE	Company-Manufacturer	3

Comment received
p 217 - p 221, 4.1 Comparison with the CLP criteria

"New data presented by BASF address, in a logical manner, key concerns expressed in the RAC Opinion of 17 March 2010. Exponent concurs that new data provide good evidence of a relevant species-related vulnerability of the rat. These data provide reasonable doubt about the relevance to humans of developmental effects seen in rats. Sufficient uncertainty remains about the induction of cleft palate (a rare malformation) at high maternally-toxic doses of epoxiconazole in rats, that a classification for developmental toxicity remains prudent. Given, however, reasonable doubt of relevance to humans, then CLP Repr. Cat 2 would appear appropriate.",," Exponent agrees that epoxiconazole would not appear to require CLP classification on the basis of post-implantation loss in rats, a model that is not relevant to humans." (Conclusions from of the expert opinion "Classification of Epoxiconazole for Developmental Toxicity" prepared for BASF SE by Simon Warren, John DeSesso and Carole Kimmel, Exponent International, UK, July 2012; the complete seven-page opinion has been uploaded as comment to the additional information report)

ECHA comment: The attachment document Classification of Epoxiconazole for Developmental Toxicity (July 2012) [Classification of Epoxiconazole for Developmental Toxicity_Exponent Int._July 2012.pdf] was submitted as a separate attachment. See attachment no. 3 in appendix.

RAC response
RAC disagrees with this position (for detailed justifications please see the opinion).

Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**
20/07/2012	Spain		MSCA	4

Comment received
Summary and discussion of reproductive toxicity

Epoxiconazole is currently listed in Annex VI of Regulation 1272/2008 as Repr. 2 (H361df: Suspected of damaging fertility) and as Xn; Repr. Cat. 3 R62 (Possible risk of impaired fertility); Repr. Cat. 3 R63 (Possible risk of harm to the unborn child) according to Directive 67/548/EC.

Regarding developmental toxicity, in the Risk Assessment Committee Opinion (March 2010) it was considered that epoxiconazole should be classified as Repr. 1B (Regulation EC No. 1272/2008) and as Repr. Cat. 2 R61 (Directive 67/548/EEC) on the basis of two main adverse effects observed in rat studies that were considered critical for the classification decision:

1) Published rat data from 2007/2008 showing post-implantation loss and resorptions in the reported absence of maternal toxicity.

2) Increased incidence of malformations as cleft palate in rats based on study data already evaluated by ECB in 1997 / 2002-2003 / 2007-2008

After an evaluation of all available information, the Spanish CA considers the convenience of maintaining as a minimum the current classification in Annex VI of epoxiconazole. However, a more severe classification could even be applied based on the following data:

1) A very high incidence of cleft palate (50,2% foetal incidence) was observed in a rat study with maternal toxicity (Hellin, 1989). This finding was also observed in most of the rat studies (Hellwing, 1990b, 1992 and 1993). Although the incidences were very low, the fact that this malformation appearsrepeatedly in the rat studies supports the conclusion that they are not of spontaneous origin and they are biologically significant.

2) This rare malformation was observed in two species. The incidence in a rabbit study was of 1.1% at 5 mg/kg bw/d (Hellwig, 1990a).

3) Although this malformation was not observed in guinea pig studies at the top dose level assessed (90 mg/kg bw/d), it can not be ruled out a possible effect at a higher dose level. The highest incidence of cleft palate in rats was observed at the dose level of 180 mg/kg bw/d.
4) The cleft palate mechanism of action has not been elucidated.

RAC response

The comment is well noted (for detailed justifications please see the opinion).

Date	Country	Organisation	Type of Organisation	Comment number
20/07/2012	Denmark		MSCA	5

Comment received

Denmark supports the classification for as Repr Cat 1B; H360. The Danish comments can be found in the attached document.

ECHA comment: The attachment document Comments to Additional Information Report, For a Substance under Classification and labelling Process, Substance Name: Epoxiconazole [Comments epoxiconazole human relevance.docx] was submitted as a separate attachment. See attachment no. 4 in appendix.

RAC response

RAC notes the support to the original classification (for detailed justifications please see the opinion).

REFERENCES: -

ATTACHMENTS RECEIVED: 4, See Appendix section.

1. ***Epoxiconazole_expert review Epoxiconazole: Evaluation of new reproductive toxicology studies by BASF and the potential regulatory impact of these new studies by Bengt R Danielsson****[Epoxiconazole_expert review attachment to SE-CA comments.doc] submitted by Sweden. Comment is not copied in the table. Comment number 1.*

2. ***Statement on the Classification of Epoxiconazole*** *[Statement on the Classification of Epoxiconazole_TSGE_July 2012.pdf] submitted by Germany. Comment is not copied in the table. Comment number 2.*

3. ***Classification of Epoxiconazole for Developmental Toxicity (July 2012)*** *[Classification of Epoxiconazole for Developmental Toxicity_Exponent Int._July 2012.pdf] submitted by Germany. Comment is not copied in the table. Comment number 3.*

4. ***Comments to Additional Information Report, For a Substance under Classification and labelling Process, Substance Name: Epoxiconazole*** *[Comments epoxiconazole human relevance.docx] submitted by Denmark. Comment is not copied in the table. Comment number 5.*

Expert review

Epoxiconazole: Evaluation of new reproductive toxicology studies by BASF and the potential regulatory impact of these new studies

27th of June 2012

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Introduction

BASF has generated new data in the process of clarifying observed fetal adverse effects in reproductive toxicological studies. In March 2010, RAC's opinion was to re-classify epoxiconazole from Repr. Cat. 2 to Repr. Cat. 1B, in agreement with Sweden's proposal, on the basis of in particular two main adverse effects in rat studies; 1) late fetal death and findings suggesting endocrine disruption potential in rats 2) very high incidence of cleft palate in rats (90% of litters affected).

The purpose of my expert evaluation is to review the public consultation documentation and comment on the validity of the arguments presented by BASF on behalf of KEMI. Since several important arguments provided by BASF are based on that signs of maternal toxicity were observed at the doses which caused adverse fetal effects, I will initially give an update of the role of maternal toxicity in reproductive toxicology. Several recent workshops have discussed this complex issue in order to get a better understanding of how to design and interpret developmental toxicity studies for chemicals and pharmaceuticals, including workshops at Society of Toxicology 2009 (Baltimore, US), the Teratology Society 2009 (Rio Grande, Puerto Rico), and the European Teratology Society 2009 (Arles, France). The outcome of these meetings are summarized in a recent "current opinion" publication (Beyer et al 2011), see reference 8. In my view, it is desirable that ECHA/RAC evaluations of importance for CLP of are in line with current opinions in the area. I will therefore below summarize relevant parts in Beyer article (8), some other relevant publications together with views expressed in regulatory guidelines on maternal toxicity of relevance to evaluate the epoxiconazole studies. Only references referred to with figures are included in the reference list, not references with name(s) plus publication year (e.g. Schneider 2010c). However, most of the "name+year" references are referred to in the BASF document.

As presented more in detail in the section entitled "Role of Maternal Toxicity in reproductive toxicology", conclusions from above mentioned conferences indicate: 1) Conducted studies and literature data *do not support that maternal toxicity* (defined as clinical signs, decreased body weight gain or absolute body weight loss of up to 15 % in rats or 7 % in rabbits) *can be used to explain the occurrence of major malformations*. The only exception is if when the fatal adverse effects clearly can be related to a maternal mechanism which is considered to be of no human relevance. 2) There is clear evidence that substantial reductions in maternal weight gain (or absolute weight loss) are linked with other manifestations of developmental toxicity than major malformations. Among these can be mentioned decreased fetal weight, and a few skeletal anomalies (e.g. wavy ribs) in rats and decreased fetal weights, post implantation loss, abortions and some skeletal anomalies in rabbits. 3) There are several examples of misinterpretation among companies, where companies incorrectly expect that regulatory authorities would not label chemicals/drugs as "teratogens/developmental toxicants" because embryo fetal adverse effects were only observed at doses also causing signs of maternal toxicity.

Current opinion on role of maternal toxicity

Although the demonstration of some degree of maternal toxicity is required in regulatory developmental toxicology studies for both pharmaceuticals and chemicals *(1-4)*, marked maternal toxicity may be a confounding factor in study design and data

interpretation. Reduced maternal body weight gain is by far the most frequently used endpoint for selecting the high dose. Other alternative endpoints of toxicity can also be taken into consideration, such as: 1) specific target organ toxicity or marked effects on haematology and clinical chemistry variables, 2) exaggerated pharmacological response, which may or may not be reflected as marked/severe clinical reactions (e.g. sedation, convulsions) and 3) marked increase in embryo-fetal lethality observed in preliminary studies *(1)*.

During the 1980s, a scientist (named Khera) proposed that maternal toxicity in rats and rabbits may have an important etiologic role in the induction of fetal adverse effects, including major malformations *(5-6)*. Maternal toxicity was defined as any type of adverse effect in the mother (e.g. adverse clinical signs, reductions in maternal body weights or maternal lethality). However, even if it's generally agreed that significant decreases in maternal food consumption and body weight gain should be avoided, Khera's hypotheses have been criticized. *Several more recent studies and analyses have not been able show an increase in malformations, even at doses causing absolute maternal body weight loss.* There are also many examples of misinterpretation of observed signs of maternal toxicity, where chemical and pharmaceutical companies expect that regulatory authorities would not label chemicals/drugs as "teratogens" or "developmental toxicants" because developmental toxicity was only observed at doses which also caused signs of maternal toxicity. However, as discussed below, evidence of maternal toxicity does not automatically negate the observation of fetal toxicity at a similar dose level. *From a regulatory perspective, companies should be able to support claims that developmental toxicity is due to maternal toxicity and that the findings have no relevance for humans (e.g., provide mechanistic or other data to support the claim).*

Is there an association between maternal toxicity and major malformations?

Based on retrospective analysis of literature data in rodents, hamster and rabbits Khera proposed that a number of effects on the offspring occurred merely as a consequence of maternally-mediated toxicity. Proposed maternal toxicity-related effects included decreased fetal body weight, external/visceral and skeletal malformations and developmental variations, and resorptions. Examples of the malformations Khera associated with maternal toxicity include exencephaly, open eye, and fused thoracic or lumbar vertebrae in mice, and fused ribs, exencephaly, and eye defects in hamsters, as well as rib, vertebral, and sternebral defects in rats and rabbits. Khera's hypothesis was that such effects were species-specific, and were seldom observed at dosages below those that were maternally toxic *(5-6)*.

As discussed by Hood and Miller *(7)*, and in the lecture by Hood at the Maternal Toxicology Symposiums in 2009 (summarized in Beyer et al., 2011*(8)*, it can be argued that it is Khera's interpretation, rather than the developmental toxicity study results themselves, that may be of concern. Khera's literature review indicated a possible association between maternal toxicity and embryo-fetal effects, but it did not establish a causal relationship between these two observations. Additional criticisms of Khera's hypothesis include the fact that his literature review was retrospective, there was a potential selection bias arising from the general tendency not to publish negative data, and the failure to adequately address maternal toxicity endpoints in the published literature of the time. In fact, Khera himself stated that in 40% of the studies he evaluated in support of his hypothesis the maternal toxicity data were "insufficient or nonexistent" *(9)*.

Studies conducted to test Khera's hypothesis that maternal toxicity commonly resulted in malformations did not find a consistent relationship for malformations in rodents. As concluded by Beyer et al *(8)*, maternal toxicity was not an effective or consistent inducer of most malformations. For example, a mouse study by Kavlock and colleagues (*10)* concluded that there was no clear direct relationship between the induction of maternal toxicity, including lethality, and the production of major abnormalities. A follow-up study with rats (*11)* reached similar conclusions: overt maternal toxicity, as defined by weight loss or mortality, was not consistently associated with a defined syndrome of adverse developmental effects in the rat. Also Chahoud et al. *(12)* concluded that maternal toxicity, defined as decrease in maternal body weight in rats, did not correlate with examined embryo/fetal parameters. Similar results were observed in a more recent feed restriction study in rats by Fleeman et al. *(13)*. Rats were offered diet either ad libitum or in restricted amounts of 20, 15, 10, and 7.5 g/day during organogenesis (gestation days (GD) 6-17). Maternal body weight gain was affected in all restricted diet groups, and in the 10 and 7.5 g group *absolute losses* in maternal body weight occurred (of 5% and 15 % respectively). Even up to a 15% maternal gestational body weight loss had no effect on embryo viability, neither was there any external, visceral, or skeletal malformation associated with maternal body weight reduction (or loss) in any of the restricted diet groups.

In rabbits, results in feed restriction studies are somewhat conflicting. Petrere et al. *(14)* did a study in which rabbits were fed ad libitum or given 150, 75, or 15 g feed/day on GD 6-18. None of the fetuses from food restricted does showed external or visceral malformations. The same results were obtained in a feed restriction study by Cappon et al. *(15)*. Rabbits were offered 150 (control), 110, 75, 55, 35, and 15 g feed/day from gestation day 7-19. Maternal body weights at the end of the feed restriction period were lower in the feed restricted group, but only the 15 g feed/day group showed a net maternal body weight loss (7%). There were no external or visceral malformations or variations, and no skeletal malformations associated with feed restriction in any group. In contrast, in a feed-restriction study by Clark et al. *(16)*, in a 15 g/day group, major and minor malformations were also observed, consisting of omphalocele, craniostenosis, clubbed forefeet, as well as cervical, thoracic, rib and sternebral malformations, variations and incomplete ossifications.

Altogether, available studies do not support that nonspecific maternal toxicity (defined as unspecific clinical signs or decreased body weight gain or absolute body weight loss of up to 15 % in rats or 7 % in rabbits) can be used to explain the occurrence of major malformations. From a regulatory perspective *(4,17-18)*, it is difficult to distinguish between those effects on *in utero* development that are attributable to direct fetal exposure to the toxicant vs. those effects that are due to, or exacerbated by, maternal toxicity. Therefore, malformations are generally considered by both chemical and pharmaceutical regulatory agencies to be developmental toxic manifestations of treatment, regardless of the cause. In some instances, the fetal findings might for some of these substances not be relevant in the human situation. However, it is the essential to demonstrate why an observed developmental effect is not relevant to humans. It is not sufficient to simply state that it is due to maternal pharmacological effects or occurs at doses which cause decreased body weight gain or unspecific signs of toxicity..

Is there a relation between reduction in maternal body weights and manifestations of developmental toxicity other than major malformations?

A study by Chernoff et al. *(19)* was undertaken to evaluate the relationship of maternal and fetal toxicity for chemicals. It constituted of an analysis of 125 developmental toxicity bioassays in the mouse, rat, and rabbit conducted by the National Toxicology Program. Although varying by species, general findings included: 1) Maternal weight reductions were associated with reduced food intake for a variety of dissimilar test agents.2) Lower fetal weights were associated with reduced maternal weight gain late in gestation. 3) The degree of fetal weight reduction is correlated with the extent of the maternal weight loss. In a substantial number of the studies, reduced fetal weights at term may, therefore, be due to maternal undernutrition caused by general toxicity rather than direct developmental insult.

The previously mentioned feed restriction studies in rats and rabbits *(13-16)* also evaluated other effects than major malformations. The results in these studies are reviewed in some detail below. In rats, *(13)* feed restriction-induced reductions in maternal body weight gain (of approximately 50% compared to ab libitum-fed rats) resulted in reductions in fetal body weights. Fetal body weights were reduced to 95%, 93%, 90%, and 76% of the control values at 20, 15, 10, and 7.5 g/day, respectively. There was also an increase in skeletal defects (wavy ribs and a reduced ossification at 7.5 g/day), but no major malformations.

In rabbits Clark et al. *(16)* found that food restriction alone caused an increase in the resorption rate (restriction to 50 g/day, 14% resorptions rate; restriction to 15 g/day, 16% resorptions rate vs. 8% in controls). They also found significant food-level related decreases in fetal weight in the food restricted groups compared to controls. Concomitant with the decreased fetal weights in the restricted groups, there was an increased incidence of fetuses with incompletely ossified skeletons. In the Petrere study, decreased maternal body weight and water consumption were significantly reduced in groups fed 15 and 75 g/day compared to ad lib or 150 g/day. Abortions were increased in the 15 g/day group, and fetal body weights were also lower in this group. Abortions and fetal loss were observed in a feed restriction study in rabbits by Matuzawa et al. (20). In the more recent rabbit feed restriction study by Cappon et al. *(15)*, (150, 110, 75, 55, 35, and 15 g feed/day), the results demonstrated that feed restriction produced substantial reductions in maternal body weight gain and developmental toxicity, such as reduced fetal weight. Fetal body weight was significantly reduced at 75, 55, 35, and 15 g feed/day (95%, 90%, 86%, and 84% of control, respectively). Other observed adverse effects were abortions and alterations in ossification. Abortion (6 out of 15 animals) occurred when feed was restricted to an amount that produced maternal body weight loss (15 g feed/day), whereas reduced fetal weight and increased incidence of fetuses with unossified sternebrae, meta-tarsals and-carpals, or caudal vertebrae were noted at feed levels of < or = 75 g/day.

Even if there is little evidence that maternal toxicity (defined as reductions in maternal body weight) is associated with major malformations, there is clear evidence that substantial reduction in maternal weight is linked with other manifestations of developmental toxicity. These manifestations include decreased fetal weights, and skeletal anomalies (e.g. wavy ribs) in rats and decreased fetal weights, post implantation loss, abortions and skeletal defects in rabbits (e.g. unossified sternebrae, metatarsals, metacarpals, or caudal vertebrae). In the EU hazard based system of categorization of chemicals, it is stated that substances should not be classified as toxic

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to reproduction if observed adverse effects are presumed to be due to maternal
toxicity. As discussed previously, nonspecific maternal toxicity is not associated with
major malformations. However, the results from the NTP program and the feed
restriction studies indicate that decreased fetal weights are due to maternal
undernutrition and concomitant reduced food intake rather than a direct developmental
insult. Consequently, the general opinion is that such substances should not be
classified as primary developmental toxicants *(8)*.

Evaluation of BASF studies in relation to current opinion on maternal toxicity

My evaluation will focus on sections where BASF discuss the relevance of the new
studies for classification (Document by Dr S Stinchcombe entitled "Epioxiconazole:
Summary on new toxicological data for the assessment of the endocrine disruption
potential and for appropriate reproduction toxicity classification" and page 208-223 in
the public document ("Relevance of new information for developmental toxicity
classification of epoxiconazole concerning the rat finding "post-implantation loss and
resorptions") ; and not review the new individual studies in detail. As mentioned in the
introduction, there are two reasons for the proposed Repr. Cat. 1B. 1) late fetal death
and findings suggesting endocrine disruption potential in rats 2) very high incidence of
cleft palate in rats (90% of litters affected).

Late fetal death and findings suggesting endocrine disruption potential in rats
Confirming studies in rats. BASF presents new GLP studies confirming non GLP
studies by Taxvig et al (2007, 2008), showing that epoxiconazole causes *fetal death*
(the BASF expression is post implantation loss/late fetal resorption) at 50 mg/kg
after treatment GD 7-21 (which includes late gestation exposure GD 16-21).

 BASF stresses that in their new studies the increased fetal death (or as BASF express
it: "post implantation loss/ late fetal resorptions") resulting from treatment with 50
mg/kg bw/d epoxiconazole *does occur in the presence of distinct maternal toxicity*
(clinical signs, statistically significant reduction in feed consumption during late
gestation, statistically significant reduction of corrected body weight gain, clear
evidence of anemia, changes of clinical chemistry parameters) [see Schneider et al.
2010a, 2010b]. In the studies published by Taxvig et al. (2007, 2008), "adjusted body
weight" was reported only in the 2007 paper (without giving any details on how the
data was adjusted); clinical signs and feed consumption data were not reported, and
hematological or clinical-chemistry examinations were apparently not performed.

My comments: Late fetal death ("late resorption") is very a very rare finding in
reproductive toxicology studies; usually early resorptions or death just after delivery
may occur for various reasons. As mentioned in the "Role of maternal toxicity"
section above, the observed signs of maternal toxicity (e.g. decreased body weight
gain and changes in clinical chemistry) can't explain the rare late fetal death. The
argument that late fetal death *does occur in the presence of distinct maternal toxicity* is
therefore of no/very limited importance.

Mechanistically oriented studies in rats at 50 mg/kg. As BASF mentions, the new
studies show that post-implantation loss / late fetal resorptions observed at 50 mg/kg
bw/d occur in the presence of a marked *decrease of the estradiol concentration* in
maternal plasma. Additional histopathological examinations revealed that

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epoxiconazole doses of 23 and 50 mg/kg bw/d administered from GD 7-18 and GD 7-21 caused a time- and dose-dependent degeneration of the placenta. The severity of the placental damage is correlated with the occurrence of late fetal resorptions. Post-implantation loss / late fetal resorptions induced by 50 mg/kg bw/d epoxiconazole treatment from GD 7-21 could be prevented by co-treatment of pregnant rats with estradiol cyclopentylpropionate [Schneider et al. 2010a, 2010b, Schneider and Rey Moreno 2011, Schneider et al 2011b.]. Overall these new data provide evidence that marked depletion of maternal estradiol levels resulting from epoxiconazole-mediated aromatase inhibition is causally related to placental damage and to late fetal death in rats. Based on the results, BASF concludes: In view of the known differences in the hormonal regulation of pregnancy between rats and humans, *the demonstrated mechanism of action for induction of late fetal resorptions in rats is considered to be of no or very limited relevance to humans.*

My comments: BASF analyses of *estradiol concentration in maternal plasma*, showing a marked decrease in estradiol in late gestation and subsequent placenta degeneration, together with the fact that co-treatment with estradiol prevented the late fetal death, is in my view a plausible mechanism for the observed late fetal death in rats.

Developmental toxicity and mechanistic studies in guinea pigs. In addition BASF had also conducted studies in guinea pig, which is a more appropriate animal model for investigations of hormonal changes in late gestation than the rat. In prenatal developmental toxicity studies, pregnant guinea pigs received epoxiconazole daily by oral gavage administration during GD 6-63 at dose levels of up to 90 mg/kg bw/d; Caesarean sectioning was performed on GD 63. Under these study conditions, there was no evidence for any treatment-related increase of post-implantation loss / late resorptions, at almost twice the dose level that caused post-implantation loss in rat developmental toxicity studies. There was no indication of any treatment-related placental damage in guinea pig studies [Schneider et al. 2011a, 2011b]. BASF had also conducted toxicokinetic and metabolism in pregnant rats and pregnant guinea pigs, showing no major differences in between the species. Based on these findings BASF conclude that the late fetal death and placenta degeneration is rat specific, and of no human relevance. BASF has also conducted studies in guinea pigs treated with up to 90 mg/kg bw/d epoxiconazole from GD 6 until the end of lactation [see Schneider et al 2011c]. None of the effects that had been observed in the rat two-generation study with 23 mg/kg bw/d epoxiconazole such as increased pregnancy duration, parturition difficulties, reduction of live litter size or reduced pup survival were seen in guinea pigs. Due to the absence of adverse effects in the guinea pig study, BASF proposes that the observed late gestation/parturition effects in effects are rat specific and of no human relevance.

My comment: BASF has conducted a number of studies guinea pigs, including mechanistic investigations, supporting the hypothesis that the late fetal death and placenta degeneration in rats is not relevant in the human situation. Hormonal levels were measured in guinea pig studies, but for unknown reasons BASF has not discussed these results in the overall discussion part (pages 208 and onwards). These results are important, especially since BASF stresses the similarities between hormone regulation in pregnancy between guinea pigs and humans. For example there was a double increase in male hormones (including testosterone) and increases in progesterone and cortisone (marked). These alterations in essential hormones, which

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potentially may cause adverse effects on the development of the offspring, are not commented at all by BASF. When discussing hormone levels in the rat, the observed alterations in hormones levels are classified as adverse effects (see page 213), while no comments are given to the significant alterations in hormones observed in the guinea pig.

Cleft palate in rats

Confirming studies in rats. In a previous study (Hellwig 1989) an extremely high incidence of cleft palate was observed at a dose level of 180 mg/kg (around 50% of fetuses or 90% of based on litter data -the variable to use when assessing teratology studies- as well as several other defects, including specific skeletal defects (mishappen sternebrae in 79% and cervical cartilage not present in 37% of the fetuses. All these fetal defects are uncommon in rats with an incidence of 0.01%, 0.06% and 0.004% respectively in BASF historical control material (untreated rats) at the time when the studies were conducted. BASF now presents new GLP studies confirming that cleft palates are induced at 180 mg/kg epoxiconazole (Schneider 2010c) in high incidences (>50 % based on litter data). An increased postnatal loss was also observed at this dose level. Signs of maternal toxicity were observed at 180 mg/and were manifested in form of reduced feed consumption, and reduced corrected body weight gain between 37-71%.

My comments: The new studies confirm that epoxiconazole is a potent teratogen in rats at 180 mg/kg. As discussed in the section "Role of maternal toxicity" section above, the observed signs of maternal toxicity (e.g. decreased body weight gain) can't explain the very high incidences of cleft palate and other manifestations observed in rats at this dose level. Even up to a 15% maternal gestational body weight loss had no effect on embryo viability, neither was there any external, visceral, or skeletal malformation associated with 15% maternal body weight loss.

Mechanistic study to investigate if cleft palate at 180 mg/kg is secondary to decrease in estradiol in rats. One group of pregnant Wistar rats were administered epoxiconazole by daily oral gavage at dose levels of 180 mg/kg from GD 6-15. Other groups received epoxiconazole (180 mg/kg orally) plus daily subcutaneous injection of 1 or 2 µg/rat/day estradiol cyclopentylpropionate. (Schnieder 2010c). The external malformations were markedly increased in all groups dosed with epoxiconazole (50-60% of the litters showed cleft palate, compared to 0% in controls); highest values were obtained in the groups receiving the high estradiol dose (2 µg/rat). In contrast, the incidence of fetal death/late resorptions decreased in estradiol supplemented groups, which is in line with the results at 50 mg/kg with and without estradiol supplementation (see subheading" Mechanistically oriented studies in rats at 50 mg/kg above).

BD Comments. The results at 180 mg/kg and 50 mg/kg, (with and without adding of estradiol-see above) clearly indicate that cleft palate and late resorptions are induced by different mechanisms. It is highly unlikely that that decreased estradiol levels (and associated placenta changes and fetal death/late resorptions) is at all related to the high incidence of cleft palate. As shown I table 3.1 on page 214, the incidence of "Late resorptions" is very similar (around 30-35%) in the 50 mg (two studies) and 180 mg/group. However, already at 50 mg/kg the measured estradiol concentrations in rats are 0.0 pmol compared to 41 pmol in untreated controls (see table 2/22 on page 48). If decreased estradiol concentrations had been the mechanism for induction of cleft

palate, it would be expected that high incidences of orofacial clefts are produced already at 50 mg/kg. However, this is not the case as shown by BASF in a series of studies (see below). Furthermore, the incidences of cleft palate were *higher* in the group receiving supplementary treatment with a high dose estradiol (compared to rats receiving epoxiconazole alone), while such supplementary treatment *prevented* fetal death/late resorptions. Altogether, generated data don't support a common mechanism for cleft palate and fetal death/late resorptions.

Follow up studies to investigate if doses of 50 mg/kg or lower cause cleft palate in rats.

In a series of new rat studies at 23 or 50 mg/kg (some of these mechanistically oriented) , BASF concludes that the isolated occurrences of cleft palate that were observed at lower dose levels in early BASF studies, most likely are incidental and not substance-related and that the NOAEL for induction of cleft palate is 50 mg/kg. Thus, a threshold for cleft palate induction exists.

My comments. It is agreed that a threshold for cleft palate induction exists; NOAEL is 50 mg/kg. However, such a threshold exists for most teratogens (basic principle in teratology-see text books). A threshold has also been shown for several other azoles which cause cleft palate as well as other malformations in rats (including ketoconazole). A special evaluation by Beate Holzum, Bayer also suggests that the teratogenicity is a class effect for azoles. Several of these azoles induce malformations only at high doses. Azoles used as drugs (including ketoconazole, itraconzole, fluconazole, voriconazole and posaconazole) have a similar pregnancy labeling despite the effects were only obtained at relatively high doses: "Due to developmental toxicity in animal studies the drug should not be used in pregnancy. Anticonception is recommended for women of childbearing potential". In my view, there is no reason that the risk assessment principles for the pesticide epoxiconazole (exposure is only associated with potential risk), should be different than for "azole pharmaceuticals" (exposure is associated with potential benefit –treatment of disease- as well as risk).

As discussed previously it is not possible to link the observed teratogenicity by epoxiconazole to maternal toxicity or decreased estradiol levels. Furthermore due to large species differences in disposition of compounds between humans and various animal species (e.g. metabolism, plasma protein binding, placental transfer, elimination pathways as well as intrinsic sensitivity of various tissues) it is not possible to establish safety margins based on mg/kg comparisons. For example, vitamin A derivatives (e.g. retinoic acid), which are highly teratogenic in humans (craniofacial malformations), cause similar teratogenicty in humans at 100 times lower doses (based on mg/kg) compared to doses which induce the same type of malformations in rodents. Much lower doses (2-5 mg/kg p.o.) of the antiepileptic drug phenytoin in humans causes cleft palate and other malformations which can be recreated in rats at much higher doses (e.g. doses up to 1125 mg/kg p.o were needed to cause high incidences of cleft palate –Rowland et al 1990). In view of these species differences it is very difficult to exclude human relevance of a clear teratogenic effect without substantial information on the mechanism underlying the effect. Unfortunately, this mechanism is still lacking for epoxiconazole, even if increasing evidence support a hERG related mechanism (see below). Lower doses can cause cleft palate (as well as malformations) in both rats (Harbison and Becker 1972) and in mice (e.g. Azarbayjani and Danielsson 2001) if the animals are dosed by the i.p. or s.c. routes on specific days of gestation. Mechanistic studies show that the malformations

are preceded by embryonic cardiac arrhythmia and hypoxia in the embryo (Danielsson et al 2005). .

Teratology studies in guinea pigs. Epoxiconazole was tested in guinea pigs at doses up to and including 90 mg/kg. No cases of cleft palate were observed in guinea pigs following exposure from GD 6-63 at dose levels of up to 90 mg/kg bw.

My comments. As previously agreed, the guinea pig is a valuable species to study effects related to hormonal changes in late gestation. However, limited data exist (including historical control data and predictive value) regarding the use of the guinea pig as species for teratogenicity testing in organogenesis (administration of the compound during early parts of gestation when organs formed). Furthermore, as discussed above it is highly unlikely that the observed teratogenicity in rats is related to decreased estradiol levels. In my view, the absence of cleft palate in a less established species for teratogenicity testing (at lower doses than those inducing teratogenicity in rats), does not eliminate concern about potential teratogenicty in humans. The risk may be if epoxiconazole exposure occurs simultaneously with other substances with hERG blocking potential (e.g. some commonly used drugs-see below).

New information for developmental toxicity classification of epoxiconazole concerning the rat finding "malformations as cleft palate"

BASF has conducted some studies to elucidate the possible mechanism underlying the teratogenicity of epoxiconalzole In the RAC Opinion from March 2010, several hypotheses for the mode of action of cleft palate formation by azoles were mentioned, specifically a) inhibition of embryonic CYP26 resulting in reduced degradation of endogenic retinoic acid which then causes dysmorphogenesis [Menegola et al. 2006] or b) blockade of the IKr potassium (hERG) channel, resulting in embryonic cardiac arrhythmia and hypoxia (e.g. Nilsson et al 2010, Danielsson et al 2007) However, RAC could not evaluate the relevance of these hypotheses because studies with epoxiconazole had been lacking.

The studies include determination of epoxiconazole concentrations in maternal plasma and in embryonic tissues following in-vivo exposure of pregnant rats, so that administered dose levels could be directly related to internal epoxiconazole concentrations [see Flick et al. 2012b]. With these data it is possible to assess the extent of placental transfer of epoxiconazole during the early phase of organogenesis. Moreover, the toxicokinetic data also allowed relating epoxiconazole effect concentrations identified in in-vitro studies to results from corresponding in-vivo studies. The administered dose levels of 50 and 180 mg/kg corresponded to maternal plasma concentrations of ca. 4.5 and 9.2 mg/L or about 14 and 28 µM, respectively, when blood sampling was performed at around Tmax after repeated dosing.

Vitamin A hypothesis.
Under in-vitro study conditions (whole embryo culture) , azoles cause dysmorphogenesis of the branchial apparatus theoretically leading to craniofacial defects (including cleft palate) at concentrations of 10 µM (e.g. Menegola et al 2006). The No-Observed-Adverse-Effect-Concentration (NOAEC) for dysmorphogenesis was 3 µM. Abnormal neural crest cell distribution was noted at and above cell culture concentrations of 30 µM in vitro. However, the in-vitro findings by Menegola could

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not be reproduced in rat embryos exposed to epoxiconazole under relevant in-vivo conditions: Female pregnant Wistar rats were exposed by repeated oral administration (gavage) from GD 6-11 to epoxiconazole at dose levels of 50, 100 and 180 mg/kg bw/d [Flick et al 2012a]. No substance-related adverse findings were observed in GD11 embryos at the dose level of 180 mg/kg bw/d when examined for signs of dysmorphogenesis. Normal neural crest cell (NCCs) migration and distribution was visualized in vivo GD 11 embryos. The reason for the discrepancy between in-vitro and in-vivo results is not known. However, a new in vivo study by Mineshima et al [2012] with ketoconazole is in line with the in-vivo findings that epoxiconazole lacks teratogenicity when exposure occurs between GD 6-11.The time-dependent teratogenicity patterns of ketoconazole and Vitamin A palmitate were compared, including the occurrence of cleft palates. Pregnant rats were exposed to single doses of either vitamin A palmitate or ketoconazole on specific gestational days between GD 8 and 15, followed by Caesarean sectioning on GD 20 and examination of rat fetuses. Ketoconazole induced cleft palates only when treatment occurred between GD 12-14, while the most sensitive time window for Vitamin A was between GD 8-10; a second window with considerably lower sensitivity for cleft palate formation by Vitamin A palmitate was between GD 12-14. Thus, the available evidence indicates that the mechanism of cleft palate formation by ketoconazole is different from that by vitamin A.

My comments: It is agreed that the received results (including absence of response during the most sensitive period and in vitro-in vivo data) do not support the retinoic acid hypothesis.

hERG hypothesis
The following text is copied from the BASF public consultation paper "The effect of epoxiconazole and of ketoconazole on HERG tail currents recorded from stably transfected HEK 293 cells (HERG-Assay) were investigated [Hebeisen 2011]. An IC_{50} value of 45.43 μM was obtained for epoxiconazole and an IC_{50} value of 2.26 μM was obtained for ketoconazole. Thus, in this investigation epoxiconazole displayed a 20 fold lower potency compared to ketoconazole. In published literature, there are no published reports of ketoconazole causing Torsade de Pointes (TdP) in humans when used alone. When comparing the IC_{50} value obtained for epoxiconazole with published data from pharmaceuticals, epoxiconazole would be considered as a weak inhibitor of HERG tail currents (Redfern et al. 2003). The relevance of this in-vitro finding with epoxiconazole remains unclear; it is difficult to follow the hypothesis that an unspecific effect such as hypoxia (speculated to be caused by repeated episodes of embryo-cardiac arrhythmia via HERG channel blockade) should elicit an increased incidence of one specific external malformation. In any case, the obtained The IC_{50} value ca. 45 μM is in support of a high-dose threshold effect (water solubility of epoxiconazole is 7 mg/L = 21 μM) that is probably of low practical relevance".

My comments
As will be discussed below, BASF seems to be unfamiliar to assess data of substances with potential to cause arrhythmia via blockade of the hERG channel. The arguments presented against the hERG hypothesis are not correct as discussed below.

A number of chemicals (both pesticides and drugs) can interfere with cardiac rhythm in humans as well as in some animal species by blocking of ion channels of importance for cardiac repolarization (particular the "promiscuous" potassium channel

IKr may interact with a large number of chemicals with aromatic rings). The IKr channel is expressed by the human-ether-a go-go gene (hERG). New studies conducted at Department of Cardiology at Karolinska Institute/Hospital, indicate that the non-innervated human, rat as well the rabbit embryonic heart during organogenesis is susceptible to IKr blocking agents, andthat embryonic heart is more susceptible than the adult heart (Cardiovasc Research, in press). Potent IKr blockers (e.g. astemiazole-Nilsson et al 2010) cause severe and long periods of irregular rhythm/periods of cardiac arrest in the rat embryo. The most common outcome following repeated dosing of potent IKr blockers during GD 6-15 in rats is high incidences of embryonic death. In the few surviving embryos, several different malformations can be induced depending on the severity and duration of the induced embryonic hypoxia (see Karlsson et al 2007, Nilsson et al 2010). Weak inhibitors (such as epoxiconazole and phenytoin) produce decreased embryonic heart rate and short episodes of irregular rhythm and cause mainly retardation of developmental processes in rodents, such as normal fusion of palate (leading to cleft palate) and growth retardation and skeletal defects correlated to retarded development after repeated dosing GD 6-15.

In contrast, if IKr blockers (both weak and potent blockers) are given in increasing doses (particularly if i.p., i.v.. or s.c. routes are used) on individual days between GD 10-14, a large spectrum of stage specific defects can be induced (e.g. Webster et al 1994, Karlsson et al 2007). The most sensitive period to induce clefts and other defects in rats by IKr blockers (single dose) is GD 11-14 which corresponds well with the data presented by Mineshima et al [2012], who have showed that ketoconazole produces cleft palate on GD 12, 13 or 14 (single dose). In this context, it is worth to mention that the rat embryonic heart starts beating on GD 10, and malformations by the IKr mechanism can only be induced after GD 9 (teratogenicity is based on that the embryo is dependent aerobic metabolism and that the embryonic heart is functional). At earlier developmental stages (GD 9 and before), the embryo is dependent on anaerobic metabolism and nutrition via passive diffusion.

Thus, the cleft palate is a likely outcome in rats for a relatively weak inhibitor, such as epoxiconazole) following repeated oral administration during organogenesis GD 6-15. Furthermore, cleft palate is not the only "specific" malformation induced by epoxiconazole (as stated above by BASF). In the study by Hellwig et al (1989) highly increased incidences of skeletal defects were also induced by epoxiconazole. The same is true for ketoconazole. Ketoconazole causes cleft palate as well as syndactyly and oligodactyly at 80 mg/kg. These digital (skeletal) malformations as well as cleft palate are also known to be consequence of "pure" hypoxia (e.g. decreased oxygen supply to the rat embryo by clamping of uterine vessels for 30 -45 minutes e.g. Leist and Grauwiler 1973,1974).

Since it is very difficult to detect and correlate sudden death in adult humans with drug induced arrhythmia (the worst consequence of IKr block), biomarkers are used to detect the potential to cause cardiac arrhythmia (QT prolongation on ECG in animal and human studies and potential to block the hERG channel in vitro). It is therefore mandatory to test IKr blocking potential in hERG test for new drugs before clinical trials. Furthermore, it is also established that effects on the heart occurs already at IC 20, and when evaluating risks the IC 20 concentration should be used (Webster et al 2002) and not IC 50.The IC 20 is around 10 µM for epoxiconazole and this concentration is lower than the measured maternal plasma concentrations following maternal administration of 50 and 180 mg/kg respectively (14 and 28 µM, respectively

at Tmax). However, the concentrations in the embryo were reported to be around 50% of the maternal concentrations (=approximately 14 and 7 µM, respectively in the embryo). These data correlate well with the fact high incidences of cleft palate was observed at 180 mg/kg, but not at 50 mg/kg. Furthermore, the potency to block hERG between different substances is less interesting (e.g. epoxiconazole vs ketoconazole); the important aspect is if a critical threshold concentration is reached for a specific substance to induce adverse cardiac rhythm disturbances which may lead to subsequent hypoxia and malformations. Otherwise, the lower doses of ketoconazole in vivo (80mg/kg compared to 180 mg/kg epoxiconazole) required to cause malformations, fit well with higher potency to block the hERG channel (=ketoconazole blocks hERG at an IC 20 of 1 µM and epoxiconazole at 10 µM).

BASF states that no cardiac arrhythmia has been directly observed for ketoconazole; a statement which is highly questionable. In humans, there are reports of Torsades de Pointes (severe cardiac arrhythmia) when ketoconazole is used alone (Mok et al 2005). The patient also showed QT prolongation several days after the ketoconazole intake. There are also several other studies indicating that azoles have caused cardiac arrhythmia (e.g.Viskin 1999, Roden 2001,Yap and Camm 2003). The risk is double and consist of both a direct risk related to their hERG blocking potential, but also because azoles (including epoxiconazole) can inhibit metabolic enzymes resulting in higher plasma of other hERG blocking substances (e.g. several commonly used drugs) as well as higher concentration of the azole itself (Dumaine et al 1998, Takemasa et al 2008). The study by Takemasa et al also shows direct correlation between acquired LQTS (Torsades de Points) via a direct inhibition of current through the hERG channel and by disrupting hERG protein trafficking within therapeutic concentrations in patients.

In conclusion, the data generated by BASF and the above mentioned literature data show that epoxiconazole blocks hERG (IC 20) and causes cleft palate at concentrations around 10 µM ; this concentration is lower than the maternal concentration obtained following repeated administration of 180 mg/kg (28 µM) supporting a hERG related mechanism. The pattern of defects, with cleft palate as the most prominent manifestation, is the expected (at least not unexpected) when a weak IKr inhibitor is administered during organogenesis (GD 6-15). The higher doses of epoxiconazole (180 mg/kg) compared to ketoconazole (80 mg/kg) required to induce cleft palate correlate with well with the higher IKr potency for ketoconazole. New studies indicate that therapeutic concentrations of ketoconazole can cause cardiac arrhythmia in the adult human and new studies indicate that the embryos across species (including the human embryonic heart), is more susceptible to react with arrhythmia than the adult heart. Altogether, the available data, including sensitive period for azoles (GD12-14 in rats) suggest IKr block as a likely mechanism underlying the similar teratogenic pattern for ketoconazole and epoxiconazole. This mechanism is of human relevance.

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Statement on the Classification of Epoxiconazole

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EXECUTIVE SUMMARY

RAC (2010) have previously recommended the classification of epoxiconazole for reproductive (developmental) toxicity in Category 1B (CLP). Subsequently, BASF have generated additional data to clarify the relevance of the effects (post-implantation loss and cleft palate seen in studies in the rat) underlying this proposal.

Additional data presented for post-implantation loss (late foetal resorption) clearly demonstrate that this effect in the rat is not only associated with maternal toxicity but is also a direct consequence of maternal toxicity. Specifically, post-implantation loss is shown to be caused by placental damage secondary to marked hormonal changes due to the inhibition of aromatase activity by epoxiconazole. Similar effects of epoxiconazole (i.e. reduced levels of circulating oestradiol, placental damage and increased numbers of late resorptions) are not observed in the guinea pig, a species considered to be a more relevant model than the rat for the developmental toxicity of aromatase inhibitors. Based on the weight of evidence which clearly demonstrates a causal relationship between maternal effects and late foetal resorption, the BASF proposal for no classification for reproductive (developmental) toxicity under CLP on the basis of post-implantation loss is considered to be justified.

Additional data presented for cleft palate demonstrate a clear association with marked maternal toxicity (hormonal perturbation and consequent placental damage) and also show that this is a high dose, threshold effect. The induction of cleft palate is not seen in the guinea pig, a species considered to be a more relevant model than the rat for the developmental toxicity of aromatase inhibitors and additional mechanistic information do not indicate the existence of a species-independent mechanism for the induction of cleft palate. While the data are less convincing than those for post-implantation loss, the weight of evidence clearly indicates the existence of a rat- specific mechanism for the induction of cleft palate which is secondary to maternal toxicity (placental damage during a critical phase of organogenesis) at high dose levels and has a threshold. The BASF proposal for classification in CLP Category 2 for reproductive (developmental) toxicity on the basis of cleft palate is therefore considered to be most appropriate.

EVALUATION

1   Background

A CLH dossier for epoxiconazole, submitted by Sweden and proposing classification under Regulation (EC) No. 1272/2008 (the CLP Regulation) in Category 1B for reproductive toxicity (pre-natal developmental toxicity), has previously been considered by the RAC. This proposal for classification was based both on findings of craniofacial malformations (cleft palate) and on a higher level of post-implantation loss seen in developmental toxicity studies performed in the rat. The proposal for the classification of epoxiconazole in Category 1B for reproductive toxicity was confirmed by the RAC; however additional relevant data has subsequently been generated by BASF to clarify the relevance of these findings. As part of a weight of evidence assessment, all data relevant to the two findings forming the basis for the proposed classification are considered, below.

2   Findings considered as a basis for the classification of epoxiconazole

a.   Post-implantation loss

New BASF studies performed in the rat with epoxiconazole confirm an increased incidence of post-implantation loss at high dose level; this finding is attributable to increased numbers of late resorptions. The duration of dosing appears to be of importance for this effect; increased post- implantation loss was not apparent in studies using a dosing period of gestation Day 6-15, whereas pronounced effects were observed in studies using extended dosing periods of up to Day 18 or 21. The studies of Taxvig *et al* (2007, 2008) considered by the RAC used dosing up to the end of gestation and observed a significant increase in post-implantation loss at a dose level of 50 mg/kg bw/d, in the apparent absence of maternal toxicity. The results of this study, considered along with other data available to the RAC at the time, were used to support the conclusion of classification in Category 1B.

It is important to note that investigations of maternal effects in standard developmental toxicity studies are relatively limited. A more recent study (Schneider *et al*, 2010a), the design of which includes much more extensive investigation of maternal toxicity, confirms the observations from earlier studies (including those of Taxvig *et al*) of increased post-implantation loss (increased late resorptions) in rats administered epoxiconazole at 50 mg/kg bw/d on gestation Days 7-18 and Days 7-21. A similar effect was not apparent in the new study at a lower dose level of 23 mg/kg bw/d. Importantly, this study also clearly identifies the presence of maternal toxicity (reduced food consumption, reduced weight gain, changes in haematological parameters indicative of anaemia), placental damage and hormonal changes including marked reductions in the level of circulating oestradiol at both dose levels of epoxiconazole.   The study therefore demonstrates that late resorption in the rat is associated with clear evidence of maternal toxicity and hormonal effects of epoxiconazole consistent with aromatase inhibition.   Additionally, a direct association is shown between the severity of placental damage and resorption; placental damage was seen at both dose levels in this study but only the more severe damage apparent at 50 mg/kg bw/d was associated with increased resorption. Further investigation (Schneider *et al*, 2010b) showed that oestradiol supplementation prevented both the placental damage and the subsequent increase in post-

implantation loss caused by treatment with epoxiconazole (50 mg/kg bw/d). An increase in post-implantation loss seen in a further study at the high dose level of 180 mg/kg bw/d (Schneider *et al*, 2010b) was similarly not seen in animals receiving oestradiol supplementation. These studies therefore show that the increased post-implantation loss caused by epoxiconazole in rat studies is secondary to placental damage caused by marked reductions in the level of circulating oestradiol.

New data therefore not only demonstrate the association of post-implantation loss with maternal toxicity but also clearly show that late resorption is a specific consequence of maternal toxicity, i.e. placental damage secondary to reduced levels of circulating oestradiol consistent with the inhibition of aromatase activity by epoxiconazole.

- *Implications of the new data for the classification of epoxiconazole*

CLP Guidance (3.7.2.4.2) states that '*developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity*'. Based on the new rat data, late resorption is considered 'unequivocally demonstrated' to be secondary to maternal toxicity. However the CLP Guidance (3.7.2.4.3) further states that '*classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally- mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1*.' The new data therefore indicate that classification for reproductive toxicity in Category 1B on the basis of post-implantation loss is not appropriate for epoxiconazole.

The rat is considered a less appropriate animal model than the guinea pig for the developmental toxicity of aromatase inhibitors due to hormonal differences (SCP, 1999). It is therefore notable that similar effects of epoxiconazole (i.e. reduced levels of circulating oestradiol, placental damage and increased numbers of late resorptions) are absent from a study of developmental toxicity in the guinea pig (Schneider *et al*, 2011b) at dose levels of epoxiconazole of up to 90 mg/kg bw/d, sufficient to cause maternal toxicity. In a pre-/post-natal developmental toxicity study in the guinea pig also performed at dose levels of up to 90 mg/kg bw/d (Schneider *et al*, 2011b), other effects characteristic of aromatase inhibition (effects on gestation length, dystocia, pup survival and development) were absent, consistent with a rat-specific response to the inhibition of aromatase activity by epoxiconazole. The CLP Regulation states that classification is not appropriate for mechanisms or modes of action not of relevance to humans.

BASF propose no classification for epoxiconazole under CLP for developmental toxicity, on the basis of post-implantation loss. This position is considered to be appropriate, and is justified based on the fact that the effects of epoxiconazole on post-implantation loss in the rat are clearly shown to be associated with and secondary to maternal toxicity. No classification is also supported by the clear absence of an effect in the guinea pig (the more relevant animal model).

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b.   Cleft palate

In studies previously considered by the RAC, the induction of cleft palate was seen in the rat at the maternally toxic dose level of 180 mg/kg bw/d. Incidences of cleft palate were noted to be variable between studies and the RAC concluded that this may be due to a degree of 'masking' by a high level of post-implantation loss seen in one study. The results of new studies performed by BASF confirm the induction of cleft palate at the high dose level of 180 mg/kg bw/d (Schneider *et al*, 2010c) but do not show similar effects at the lower (but still maternally toxic) dose levels of 23 and 50 mg/kg bw/d (Schneider *et al*, 2010a,b).   The potential confound effect of 'masking' at lower dose levels is eliminated in the new data through the use of oestradiol supplementation, which protects against post-implantation loss caused by epoxiconazole in the rat.   The weight of evidence from all rat studies therefore demonstrates that the induction of cleft palate by epoxiconazole is associated with marked maternal toxicity and, additionally, indicates a clear threshold level for this effect.

The high incidence of cleft palate seen at the markedly toxic dose level of 180 mg/kg bw/d epoxiconazole was unaffected by oestradiol supplementation (Schneider *et al*, 2010c), however levels of circulating oestradiol remained markedly lower in the oestradiol supplemented groups. Measurements of placental weight indicate that a significant degree of placental damage occurred in the epoxiconazole-treated groups and was also apparent in the oestradiol supplemented groups.

Compared to the clear evidence in the rat, findings of cleft palate are notably absent in the guinea pig (Schneider *et al*, 2011b), a species in which comparable metabolism and toxicokinetics have been demonstrated. While the rat is not considered to be an appropriate animal model than the guinea pig for the developmental toxicity of aromatase inhibitors due to hormonal differences (SCP, 1999), it is also important to exclude the possibility (as suggested in the RAC conclusion) that cleft palate in the rat may be induced though a non species-specific mechanism.   This possibility has been investigated in new mechanistic studies provided by BASF. While exposure of cultured rat embryos to epoxiconazole *in vitro* was shown to cause abnormal neural crest cell migration and branchial dysmorphogenesis (Menogala, 2012), similar effects were not apparent *in vivo* following exposure to equivalent concentrations of epoxiconazole (Flick *et al*, 2012a).   Investigation of the potential of epoxiconazole to block HERG channels (Hebeisen, 2011) showed only a weak effect and at concentrations unlikely to be relevant *in vivo*.   Other published data (Mineshima *et al*, 2012) demonstrate that the induction of cleft palate by ketoconazole and retinyl palmitate occurs through distinct mechanisms.

▪   *Implications of the new data for the classification of epoxiconazole*

CLP Guidance (3.7.2.4.2) states that '*developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity*'. The RAC have previously concluded that '…*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*.'

The results of recent studies confirm the induction of cleft palate by epoxiconazole in the rat at dose levels associated with marked maternal toxicity. Cleft palate was shown at 180 mg/kg bw/d which caused a marked reduction in circulating oestradiol levels and placental damage, but was not observed at dose levels of 50 mg/kg bw/d and lower, at which maternal toxicity was still apparent. The available data therefore indicate a clear threshold for the induction of cleft palate by epoxiconazole in the rat; the potential confounding factor of 'masking' having been eliminated through the protection against late resorption afforded by oestradiol supplementation.   The existence of a threshold is consistent with cleft palate being secondary to maternal toxicity. The effect is very likely to be due to placental damage during a critical period of organogenesis, secondary to the rat-specific hormonal effects of epoxiconazole.

The CLP Guidance (3.7.2.4.3) further states that '*classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1.*' The induction of cleft palate by epoxiconazole is not seen in the guinea pig, which is considered to be the more relevant species.

Additionally, mechanistic investigations have failed to demonstrate that epoxiconazole causes cleft palate through a species-independent mechanism, as suggested by RAC. These included effects on neural crest cell migration and branchial dysmorphogenesis in vitro and in vivo, investigations of effects on the HERG channel in vitro as well as literature data on the induction of cleft palate by retinyl palmitate and the structurally-related azole substance, ketoconazole. The results of these mechanistic studies therefore further support the theory that the induction of cleft palate in the rat are specific to this species and occur secondary to maternal toxicity

Classification in Category 2 is appropriate for substances with '*…some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect…on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects*'.

For epoxiconazole, findings in the rat but not in the guinea pig are considered to constitute 'some evidence' of an effect in experimental animals, rather than 'clear evidence', a criterion for classification in Category 1B.   The absence of findings in the guinea pig (the more relevant species) and the additional mechanistic data means that the available evidence is 'not sufficiently convincing' to warrant classification in Category 1B.   While effects in the rat are not conclusively demonstrated to be a secondary consequence of maternal toxicity, the evidence is sufficient to conclude that this is the most likely mechanism.   There is also a clear threshold and also a clear association in this species with maternal effects including perturbation of circulating hormone levels and placental damage. The weight of evidence is therefore consistent with the induction of cleft palate in the rat as a direct consequence of maternal toxicity.

BASF conclude that there is a 'high likelihood' that cleft palate occurs due to (rat-specific) placental damage secondary to hormonal changes during a critical period of organogenesis and that data from the guinea pig are more relevant. Classification in CLP Category 2 is therefore proposed on the basis of cleft palate. While a degree of doubt remains over the precise mechanism in the rat, classification in Category 2 is considered to be most appropriate for epoxiconazole as data clearly indicate that the induction of cleft palate is secondary to maternal toxicity in the rat. Effects are not seen in the more relevant animal model leading to the conclusion that the effects in the rat are most likely occur through a species-specific mode of action, mechanistic investigations not having clearly demonstrated the existence of a mechanism of relevance to humans. The data are not sufficiently
convincing to warrant the classification of epoxiconazole in Category 1B.

3   Overall conclusion

Based on a weight of evidence analysis, the classification of epoxiconazole in Category 2 for reproductive (developmental) toxicity under CLP is appropriate on the basis of findings of cleft palate in the rat. No classification is required for findings of post-implantation loss in the rat.

David J Andrew PhD DABT ERT

TSGE

16th July 2012

E^xponent®

Classification of Epoxiconazole
for Developmental Toxicity
(July 2012)

E^{x}ponent®

**Classification of Epoxiconazole for
Developmental Toxicity
(July 2012)**

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Classification of Epoxiconazole for Developmental Toxicity (July 2012)

Executive Summary

Exponent has reviewed a submission to RAC by BASF, to advise if BASF conclusions on classification are appropriate. Given the short timelines available, Exponent's evaluation has concentrated mostly on the key themes of the submission.

Exponent agrees that new data submitted by BASF are consistent with a species-specific vulnerability of the rat to epoxiconazole. Key conclusions stated in the RAC Opinion of 17 March 2010 have been addressed in a logical manner. The new data constitute reasonable doubt about the relevance to humans of developmental effects seen with epoxiconazole in rats. The relevance of these new data provide "reasonable doubt" about the concerns stated in the RAC Opinion of 17 March 2010, and appear to meet criteria for classification with no more than CLP Repr. Cat:2.

Introduction

Exponent has been contracted by BASF SE to offer an expert opinion on the appropriate classification of epoxiconazole for developmental toxicity, including an assessment of the robust data summaries and argumentation submitted by BASF SE to support classification with respect to developmental toxicity: CLP Repr. Cat. 2

Data Sources and Methods

The basis for Exponent's assessment was three documents available on the ECHA website:

- BASF's submission of new data and proposal for classification:
  http://echa.europa.eu/documents/10162/13626/clh_ed_request_epoxiconazole_report_en.pdf
- The RAC opinion of 17 March 2010:
  http://echa.europa.eu/documents/10162/13579/rac_opinion_epoxiconazole_en.pdf
- The RAC Background Document 17 March 2010:
  http://echa.europa.eu/documents/10162/13579/rac_bd_epoxiconazole_en.pdf

Exponent specialists in developmental toxicity and EU classification considered the scientific basis for the RAC opinion; then considered if the data contained within the BASF submission might meaningfully modify the scientific basis of the RAC opinion. Within the time constraints, this consideration has necessarily focused on the principles of the argument, checking detailed individual study summaries only in certain essential areas. This consideration is restricted only to classification for effects on development.

Evaluation

With respect to developmental toxicity, the RAC Opinion cited two critical issues resulting in a CLP Repr. Cat. 1B recommendation:

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- Post-implantation loss and resorptions;
- Malformations as cleft palates

For both of these issues, the RAC Opinion emphasized:

- clear evidence of the effect, which was

- not considered to be a secondary non-specific consequence of other toxic effects; and

- in the absence of relevant mechanistic information "it cannot be concluded that there is a doubt about the relevance for humans".

With respect to post implantation loss and resorption:

BASF provides substantial evidence from studies evaluating maternal effects in great detail that late resorptions in rats occur in the presence of distinct maternal toxicity. The new data provide important clues as to the mechanism of these effects in rats by providing information on hormonal changes as a result of epoxiconazole-mediated aromatase inhibition and concomitant degeneration of the placenta correlated with the occurrence of late resorptions.

The primary BASF position is that findings in rats are species-specific, occurring in rats which have a different hormonal control of late pregnancy than humans. This position is supported by data from guinea pigs which are more comparable in this respect to humans. BASF demonstrates with substantial data an absence of effect on placental degeneration, and on post-implantation loss and late resorptions in the guinea pig, even at higher doses given over a longer period of time than those showing effects in rats. Reference is also made to the precedent of an Opinion of the Scientific Committee on Plants (for a different chemical), that the guinea pig model seemed to be the model of choice for defining the level of risk for the human for the effect of aromatase inhibition.

Exponent confirms the view that the guinea pig is a model more representative of human pregnancy than the rat. Exponent agrees with the position that these findings are likely species-specific to small rodents, and that the new data clearly represent "mechanistic data that raises doubt about the relevance of the effect to humans". For these reasons, Exponent agrees that epoxiconazole would not appear to require CLP classification on the basis of post-implantation loss in rats, a model that is not relevant to humans.

With respect to malformations and cleft palate:

The RAC Opinion notes a high incidence of cleft palate at doses that are evidently toxic, but also repeated observations of isolated cleft palates in rats at doses without maternal toxicity. The RAC Opinion identifies induction of cleft palate as a clear developmental effect of epoxiconazole, which cannot be attributed to maternal toxicity such as decreased food consumption or bodyweight gain and cannot be considered secondary to other maternal toxic effects.

BASF reasons that induction of cleft palate shows a clear threshold effect in rats, with a NOAEL at around 45-60 mg/kg bw/day; above this threshold maternal toxicity is evident. Cleft palate was seen only at 180 mg/kg bw/day and therefore occurs at clearly maternally toxic doses. The possibility of teratogenesis being "masked" by resorption of affected fetuses, at a

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dose where resorption might be relevant, was addressed using concurrent treatment with estradiol cyclopentylpropionate to maintain pregnancy. Two incidences of isolated cleft palate in rats at lower doses, each in a different study within the epoxiconazole study set, were fetuses with multiple external malformations, hence a cause other than epoxiconazole is plausible. New data are provided to address specific possible mechanisms of teratogenesis as cited in the RAC opinion, which are indicated to be without relevance. No malformations were found in a study of guinea pigs. The known differences in hormonal regulation between rats and guinea pigs, and placental damage occurring only in rats suggest cleft palate to be a species-specific finding of limited relevance to humans. However, given remaining uncertainties BASF proposes a classification of CLP Repr. Cat 2.
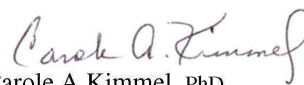
Exponent finds it plausible that a species-specific mechanism is relevant. Placental damage in the rat, not evident before the new data and not expected to occur in humans, may plausibly be associated with cleft palate, particularly at high doses. The highest dose tested in the guinea pig, at which no cleft palates were demonstrated was, however, lower than the dose seen to cause cleft palate in the rat. The incidence of two fetuses with cleft palate at lower doses may feasibly be attributable to spontaneous multiple malformations; the RAC opinion however cites within the epoxiconazole data set, in total four isolated incidences of cleft palate in the rat at low doses. Given the demonstration of a marked species-specific vulnerability of the placenta, and adequate tabulation of an otherwise clear threshold, Exponent considers that within an extensive study set for epoxiconazole, the four instances of isolated cleft palate at lower doses, each in different studies and two in fetuses that presented with other abnormalities, are not "clear evidence" of an effect in the absence of significant maternal toxicity. There is then no basis for a more severe classification than CLP Repr. Cat 2.

Conclusion:

New data presented by BASF address, in a logical manner, key concerns expressed in the RAC Opinion of 17 March 2010. Exponent concurs that new data provide good evidence of a relevant species-related vulnerability of the rat. These data provide reasonable doubt about the relevance to humans of developmental effects seen in rats. Sufficient uncertainty remains about the induction of cleft palate (a rare malformation) at high maternally-toxic doses of epoxiconazole in rats, that a classification for developmental toxicity remains prudent. Given, however, reasonable doubt of relevance to humans, then CLP Repr. Cat 2 would appear appropriate.

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July 20, 2012

Comments to Additional Information Report, For a Substance under Classification and Labelling Process, Substance Name: Epoxiconazole.

Generally, the argumentation given against classification for developmental toxicity of epoxiconazole based on the adverse effects post-implantation loss and late resorptions in rats is that the effects are "rodent-specific …, i.e. via a mechanism that is not relevant for humans" (p. 218).

We do not agree with this line of argumentation for dismissing relevance for humans and find that two very important questions need to be addressed, i.e.
1) Is aromatase inhibition relevant for humans?
2) If yes, which adverse effects can be expected in humans?

Below we give our input to these two questions. Due to limited time for the commenting during the summer vacation period this will include only the most important messages. Also, due to the time constraints comments concerning malformations as cleft palates are not included here.

Is aromatase inhibition relevant for humans?
Consistent with the fundamental biological importance of estrogen synthesis, the aromatase complex is highly conserved among vertebrates (Conley and Walters, 1998).

The two studies in guinea pigs also show signs of aromatase inhibition during pregnancy as similarly increased levels of testorone and androstenedione on GD 63 are found. On both p. 70 and p. 92 it is mentioned that these findings 'may be related to aromatase inhibition". Thus these new results shows that the aromatase inhibition observed in rats occur also in guinea pigs.

Unlike the studies in rats, estradiol appeared not to decrease significantly in guinea pigs. However, the control values on GD 63 in the two studies (tables 2/30 and 2/41) are very different, i.e. they are listed as ~159+16 nM and ~19+58 nM, respectively. Also, the variation in the control group in the 2nd study is much larger than the mean which is quite unusual and not seen in the dosed animals in the same study. Given these uncertainties, we find that the estradiol levels cannot be evaluated based on the available values.

For several drugs used for treatment of severe diseases in humans the intended mechanism of action is aromatase inhibition demonstrating that this mechanism is also relevant for humans (EMA website, www.ema.europa.eu).

Which adverse effects of aromatase inhibition can be expected in humans?

It has been recognized for many years, that excessive levels of androgens (testosterone, androstenedione) during development cause the external female genitalia to develop in a male direction, i.e. induce female pseudohermaphroditism (e.g. Sadler 1985 and Becker 1995). The masculinization of the external genital may vary from enlargement of the clitoris to almost male genitalia. In addition, androgen excess may prematurely virilize the external genitalia of the male fetus and virilize a woman during the pregnancy of an affected fetus of either sex (Pinsky et al 1999).

This risk during pregnancy is clearly recognized by the European Medicines Agency (EMA) concerning drugs where the active substance is an aromatase inhibitor (e.g. letrozole in Femara). EMA (2012) concludes that "there are safety reasons for not treating premenopausal or even perimenopausal women with letrozole. Letrozole inhibits the enzyme involved in the synthesis of oestrogens, which are required for proper embryo and foetal development. Letrozole is thus predicted to have a potential for adverse effects on the embryo-foetus, as confirmed by studies in pregnant rats and rabbits " and also that "postmenopausal status must be fully established before initiation of and during treatment of Femara and Femara should not be used in women when postmenopausal status is not fully established."

Thus, adverse effects of aromatase inhibition on sexual development can be expected in both rats, guinea pigs and in humans. With that in mind, it is surprising that no specific endpoints for effects on sexual development were included in the newly performed studies in rats and guinea pigs.

Conclusion
We find that although the adverse effects investigated so far in rats (i.e. dystochia, late resorptions) may or might not be directly relevant for humans, the severity and frequency of these effects signals a major disturbance of the endocrine system that most likely involve aromatase inhibition as a key mechanism of action. We find that this seen in combination with the clearly recognized potential for adverse effects due to aromatase inhibition in humans is a sufficient basis for classification of epoxiconazole for developmental toxicity in Category 1B ("Presumed human reproductive toxicant")

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