ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Annex 1 to

the RAC Opinion on toxicity to reproduction of

Epoxiconazole

Opinion of the Committee for Risk Assessment on a dossier proposing harmonised Classification and Labelling at Community level17 March 2010

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Adopted

28 November 2012

17 March 2010
CLH-O-0000000630-85-05/F

Opinion of the Committee for Risk Assessment on a dossier proposing harmonised Classification and Labelling at Community level

In accordance with Article 37 (4) of the Regulation (EC) No 1272/2008 ("the CLP Regulation"), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling of

Substance Name:	***Epoxiconazole***
EC Number:	***406-850-2***
CAS Number:	***133855-98-8***

The proposal was submitted by ***Sweden***
and received by RAC (co-) rapporteur on ***27 January 2009***

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/doc/consultations/cl/clh_axvrep_sweden_epoxiconazole.pdf*** on ***23 February 2009***. MSCAs and parties concerned were invited to submit comments and contributions by ***9 April 2009***.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Annick Pichard***
Co-rapporteur, appointed by RAC: ***Jose Tarazona***[1]

The opinion takes into account the comments of MSCAs and parties concerned provided in accordance with Article 37 (4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling has been reached on 17 March 2010, in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex II.

The RAC Opinion was adopted by ***consensus.***

[1] Co-rapporteurship ceased on August 16, 2009 as the Member was appointed as RAC Chair.

OPINION OF RAC

RAC adopted the opinion that ***Epoxiconazole*** should be classified and labelled as follows:

Classification & labelling in accordance with Directive 67/548/EEC
Classification: Carc. cat. 3; R40 **Repr. Cat.2; R 61** **Repr. Cat. 3; R 62** **N; R51-53**
Specific concentration limits: None
Notes: None
Labelling: Xn; N **R: 40-61-62-51/53** **S: (1/2)-45-53-61**

Classification and labelling in accordance with the Classification, Labelling and Packaging Regulation
Classification: Carc. 2 - H351 **Repr. 1B - H360Df** **Aquatic Chronic 2 - H411**
Specific concentration limits: None
M-factors: None
Notes: None
Labelling: Danger **GHS08, GHS09** **H351, H360Df, H411**

Opinion on justification for need for action at Community level

Sweden has submitted a proposal to revise the classification of epoxiconazole for effects on development from Repr. Cat. 3; R63 to Repr. Cat. 2; R61. Two studies not previously considered at TC C&L in 2003 are presented:

- Taxvig, C., Hass, U., Axelstad, M., Dalgaard, M., Boberg, J., Raun Andeasen, H. and Vinggaard, AM. 2007 Endocrine-disrupting activities in vivo of the fungicides tebuconazole and epoxiconazole, Toxicological Sciences 100(2), 464-473.
- Birkhøj Kjaerstad, M., Raun Andeasen, H., Taxvig, C., Hass, U., Axelstad, M., Metzdorff, S. and Vinggaard, AM. 2007 Effects of azole fungicides on the function of sex and thyroid hormones. Pesticides Research No 111, Danish Environmental Protection Agency.

In addition, during the public consultation comments received indicated that an additional scientific paper had been published on the reprotoxicity of epoxiconazole, which was not included in the Annex XV dossier of Sweden. The study has been included in the background document so that all relevant data were considered:

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

It should also be noted that additional information was provided by Ulla Hass, one the author of Taxvig studies at RAC 9 meeting and was included in the background document.
Moreover two more published studies (Tiboni 2009, Albrecht 2000) which were presented by an advisor of a RAC member at RAC 9 were also considered and included in the scientific justification.

SCIENTIFIC GROUNDS FOR THE OPINION

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

- Post implantation loss and resorptions
- Malformations as cleft palates

<u>Post-implantation loss</u>

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

By oral route, whereas no significant increase in post-implantation loss was observed in studies in which rats were exposed to 45 mg/kg/d epoxiconazole (Hellwig 1990b) and to 180 mg/kg/d (Hellwig 1989) from gestation days (GD) 6 to 15, a large increase of post-implantation loss was observed in Schneider 2002 at the same dose of 180 mg/kg/d with an exposure partially extended to the end of gestation (GD 6-19). Resorptions were mainly identified as late resorptions.

In Taxvig 2007 and 2008, in which exposure was entirely extended to the end of gestation (GD7-21), a significant increase in post-implantation loss was observed at 50 mg/kg/d and consisted in late and very late resorptions.
No effect is observed in rat by the dermal route up to 1000 mg/kg/d (Hellwig 1993).
An increase in post-implantation loss was also observed at the highest dose by the oral route in rabbits in presence of maternal toxicity (Hellwig 1990a) and consisted mainly of early loss in contrast to rats.

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

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

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

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

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

Cleft palates

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

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

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

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

Overall, RAC considers that based on a weight of evidence approach and considering the specificity and the spontaneous infrequency of this malformation otherwise commonly seen

with triazoles, the induction of a high incidence of cleft palates in the presence of maternal toxicity (Hellwig 1989) and the repeated observation of isolated cleft palates in rats at doses without maternal toxicity enable **a clear identification of cleft palate as a developmental effect** of epoxiconazole. It is considered that induction of cleft palates cannot be attributed to maternal toxicity such as decreased food consumption or reduced body weight gain and **it cannot be considered secondary to other maternal toxic effects**.

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

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

Overall conclusion

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

Additional information

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

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

However, RAC was tasked only with providing an assessment of the proposal from Sweden and data gathered during the public consultation.

Consequently, in accordance with RAC procedures, RAC did not take into account of these studies, given that the information about them was presented after the public consultation has ended.

The background document, attached as Annex I, gives the detailed scientific grounds for the Opinion.

ANNEXES:

Annex 1	Background Document (BD)[2]
Annex 2	Comments received on the Annex XV report and response to comments provided by the dossier submitter (excl. confidential information)

[2] The Background Document (BD) supporting the opinion contains scientific justifications for the CLH proposal. The BD is based on the CLH report prepared by a dossier submitter. The original CLH report may need to be changed as a result of the comments and contributions received during the public consultation(s) and the comments by and discussions in the Committees.