Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Prothioconazole (ISO); 2-[2-(1 -chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione

EC Number: -
CAS Number: 178928-70-6

CLH-O-0000001412-86-269/F

Adopted
15 March 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PROTHIOCONAZOLE (ISO); 2-[2-(1-CHLOROCYCLOPROPYL)-3-(2-CHLOROPHENYL)-2-HYDROXYPROPYL]-2,4-DIHYDRO-3H-1,2,4-TRIAZOLE-3-THIONE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: prothioconazole (ISO); 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione
EC number: -
CAS number: 178928-70-6
Dossier submitter: United Kingdom

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	Germany		MemberState	1
Comment received				
The DE-CA agrees with the proposal of classification for environmental hazards as Aquatic acute 1 (H400), Aquatic chronic 1 (H410) and the acute M-factor of 10, chronic M-factor of 1. Since harmonised precautionary statements are not part of Annex VI part 3 table 3 of the CLP Regulation, the precautionary statement P273 should be deleted in the CLH dossier.				
Dossier Submitter's Response				
Thank you for your support. We agree that P273 should be removed from Table 5 of the CLH dossier.				
RAC's response				
Thank you for your comment. RAC notes the support for the proposed environmental classification.				

Date	Country	Organisation	Type of Organisation	Comment number
15.06.2018	Germany		Individual	2
Comment received				
Given our own extensive theoretical studies on cytochrome P450 enzymes over the past two decades, I have read the expert statement on prothioconazole (PTZ) with great interest. The applied computational methods and protocols are sound and state-of-the-art, the quantum-chemical computations have been performed competently, and the				

interpretation of the computational results is convincing. The computational study clearly elucidates the major differences in the atomistic interaction mechanism between the P450 enzyme CYP51 on the one hand and PTZ and PTZ-dethio on the other hand. This provides strong and convincing evidence that PTZ does not cause the toxicity that is observed for classical triazoles because of their interaction with cytochrome P450 enzymes.
Dossier Submitter's Response
Noted, thank you.
RAC's response
Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Belgium		MemberState	3
Comment received				
BE CA thanks UK CA for their CLH proposal.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Belgium		MemberState	4
Comment received				
Regarding fertility, BE CA is of the opinion that the observed effects should be discussed. In particular, in a two-generation study in rat, effects on fertility associated with maternal hepatotoxicity were observed after 750 mg/kg bw day, including decreased number of oestrous cycles, implantation sites, litter size in F0 and F1 generations. These effects might warrant a Repr. 2 H360f classification Adverse effects have also been observed. In particular, microphtalmia and short 14th rib have been reported in fetuses in association with maternal toxicity from 80 mg/kg bw/day in a developmental toxicity study in rat. Supernumerary 14th rib was also observed in another developmental toxicity study also at dose causing maternal toxicity (750 mg/kg bw/day). Finally, in rabbit, increased post-implantation losses and decreased fetal weight were reported, again with toxicity in dams. Overall, the observations of toxic effects in fetuses in three different studies, including two different species, should be carefully assessed, maybe leading to a Repr. 2 H360d classification				
Dossier Submitter's Response				
All findings in the reproduction and developmental toxicity studies have been thoroughly evaluated and considered against the classification criteria in the CLH report, as outlined below. <u>Fertility</u> In both reproduction studies, there was marked parental toxicity in the high-dose groups (500 mg/kg bw/d in the range-finding study; 750 mg/kg bw/d in the main study). Urine				

stains were common to both studies, and in the main study dehydration (albeit in a small number of animals) was also recorded. Kidney toxicity with severe dehydration and increased water consumption was a feature of prothioconazole administration in several repeated-dose studies in rats at similar doses and in the developmental toxicity studies, which eventually led to death after prolonged administration for one to two years. Although water consumption was not measured in the reproduction studies, the urine staining was likely to be a sign of effects on the kidney and water homeostasis systems, consistent with the findings of the repeated-dose toxicity studies. Histopathological changes in the kidneys were also observed at 750 mg/kg bw/d in the main study. It is therefore reasonable to surmise that the observed urine stains and dehydration were manifestations of kidney dysfunction; furthermore, cortical nephrosis was observed at this dose. The dossier submitter therefore considers that 750 mg/kg bw/d was a clearly maternally-toxic dose. Please also see the response to comment number 14.

Other treatment-related parental findings in the high-dose group included increased food consumption and a decrease in body-weight gain (indicative of a reduction in food utilisation efficiency), increases in organ weights, particularly the liver and kidneys, and histopathological changes in the liver. A slight decrease in body weight was also evident in males at 100 mg/kg bw/d, as was a decrease in thymus weight in females.

With regards to fertility, there were no treatment-related effects on either mating or fertility indices in either generation; slight increases in the time to insemination were not statistically significant. Pregnancy outcome was not affected by exposure to any dose. Slightly reduced mean numbers of implantation sites and litter sizes in the high-dose group were within the historical control data for the F_0 generation and were not likely to be real effects or were a consequence of maternal toxicity, as was the slight increase in the duration of gestation in F1 dams (not associated with clinical signs or deaths in either dams or pups).

Prothioconazole did not adversely affect pre-natal or post-natal pup viability at any dose. Toxicity to pups in both generations consisted of clinical signs (urine staining and salivation) and retarded growth only at 750 mg/kg bw/d, secondary to general offspring toxicity. There was no evidence of a specific effect on development.

In conclusion, in the available studies prothioconazole did not demonstrate a specific effect on reproduction. Minor changes to some parameters occurred only in the high-dose group and were secondary to the relatively severe maternal toxicity that was induced at this dose. Therefore, no classification for adverse effects on sexual function and fertility is warranted.

<u>Development</u>
In the first oral study in Wistar rats (sub-strain Hsd Cpb:WU), a treatment-related increase in the incidence of foetuses and litters with microphthalmia was reported at the very high dose of 1000 mg/kg bw/d. Overt maternal toxicity, consistent with effects on kidney function and water / electrolyte homeostasis, was evident at this dose and also at the mid-dose level of 500 mg/kg bw/d. Effects on maternal body-weight were also apparent at these doses. An analysis of individual animal data showed that the dams with the lowest body-weight gains and feed intake were those that produced pups with microphthalmia; and that the lowest-weight foetuses (as a secondary effect to maternal toxicity) were in the affected litters. Considering this data and the high spontaneous occurrence of this malformation in this strain of rat, the applicant under Regulation 844/2012 concluded that the induction of microphthalmia was not a specific

developmental effect of prothioconazole administration, but was rather an exacerbation of the background incidence as a result of maternal toxicity. To demonstrate the plausibility of this hypothesis, an inhalation study in the same strain with an irritant, non-teratogenic substance was submitted; the occurrence of microphthalmia at a higher incidence than that in the prothioconazole study, with partial abrogation of the effect upon oxygen supplementation (reduced hypoxia), established the non-specific nature of the finding.

Further corroboration was provided by a supplementary oral study in a strain of rat with a virtually zero background incidence of ocular malformations (Wistar Hanover, Crl:WI(HAN)). Even at doses up to 750 mg/kg bw/d, which were severely maternally toxic, no cases of microphthalmia or other ocular malformations were recorded. In contrast, a positive control substance tested in the same strain demonstrated the sensitivity of the system to specific developmental toxicants. Taking into account all the evidence, the dossier submitter concludes that prothioconazole did not directly and specifically induce malformations in rats, but as a result of maternal toxicity resulted in a secondary, non-specific increase in microphthalmia in a strain of rat with a relatively high spontaneous incidence of this malformation.

In both the rat studies, increased incidences of rudimentary 14th ribs were reported in the high-dose groups, although the increase at 750 mg/kg bw/d in the supplementary study was marginal. As shown by the historical control data for both rat strains, this is a very common variation (i.e, a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health). It is also notable that in the range-finding experiment for the first study, the incidence of this variation in the mid-dose group was double that in the high-dose group, again demonstrating that this finding is common and often has a large inter-group variability. Furthermore, rudimentary supernumerary ribs (as opposed to full supernumerary ribs) in rats are generally regarded to be of low toxicological and biological relevance, since they do not persist beyond post-natal day 40 to 60 and do not appear to give a reliable prediction of hazard in human development. An increased incidence of rudimentary 14th ribs is also associated with maternal stress. In the supplementary study, there was clearly not a treatment-related increase in supernumerary ribs at the low- and mid-dose levels of 20 and 80 mg/kg bw/d, respectively. In the first study, however, there was an apparent dose-related increase at all doses (80, 500 and 1000 mg/kg bw/d). The extensive historical control data showed that the statistical significance of the increases in the low- and mid-dose groups was confounded by an unusually low incidence in the concurrent control group. Furthermore, the incidences at 80 and 500 mg/kg bw/d were well within the historical control range.

In the first rat study, indicators of delayed development comprised reduced foetal weights, renal pelvis dilatation and delayed skeletal ossification only at 1000 mg/kg bw/d; the dossier submitter concludes that they were secondary to the severe maternal toxicity at this dose and thus were not indicators of specific developmental toxicity. Ossification changes were inconsistent in the oral rabbit study; the main treatment-related observation in this study comprised abortions, total litter losses and reduced foetal weights at the high-dose level of 350 mg/kg bw/d, which were secondary to the very severe maternal toxicity at this dose (death, body-weight loss, reduced body-weight gains, reduced food consumption). There was no indication of specific developmental toxicity in rabbits.

Overall, prothioconazole resulted in developmental toxicity only at severely maternally-toxic doses: there was no evidence of an effect on development in the absence of maternal toxicity. Please also see the responses to comment 14.

Following oral high-dose administration of the active substance to rats, increases in microphthalmia (one, rat strain exhibiting a high spontaneous incidence of this malformation) and supernumerary ribs (both rat strains) occurred; therefore, oral administration of prothioconazole was associated with developmental toxicity. On this basis, a case for classification in category 2 could be made. However, these developmental effects were only reported at extremely high doses (1000 mg/kg bw/d for both findings in the first rat study; marginal change in the incidence of supernumerary ribs at 750 mg/kg bw/d in the second study); neither finding was increased at the next dose of 500 mg/kg bw/d (in the first study), at which maternal toxicity was still evident but less severe. Rudimentary supernumerary ribs are a very common variation that have no effect on survival and do not persist post-natally; as such, an increase in this finding at doses that also cause maternal toxicity does not support classification. Prothioconazole's involvement in the induction of microphthalmia in rats is not clear: there is no known mode of action for this substance by which this effect might have been expressed, and the finding was only reported in one study and when administered at a very high dose. The most likely explanation for both findings was that prothioconazole was not directly, specifically responsible; rather, the systemic effects suffered by the dams, with consequences on the growth and development of the foetuses, resulted in disruptions to normal development and an increase in spontaneous findings. This supposition is supported by the absence of any cases of microphthalmia in the second rat study. No treatment-related malformations or variations were reported in an oral rabbit study at doses that were excessively toxic.

As explained in the CLH report, the findings in the two developmental toxicity studies in rats with prothioconazole could support no classification for developmental toxicity, or classification in category 2. The main considerations are the uncertainty around the direct causative involvement of this substance in the occurrence of microphthalmia in only one of the studies; the extremely high dose at which this occurred; and the nature and reversibility of the supernumerary ribs (a common variation) in association with maternal toxicity. On balance, taking into account all the available evidence, the dossier submitter concludes that the criteria for classification in category 2 are not met and proposes not to classify prothioconazole for adverse effects on development.

RAC's response

RAC acknowledges that this is a case between no classification and classification in category 2, for both sexual function and fertility and development, that will be discussed in the ODD.

Date	Country	Organisation	Type of Organisation	Comment number
27.05.2018	Germany		Individual	5

Comment received

I am a consultant in reproductive toxicology with more than 35 years of experience in this field. As a consultant for Bayer I reviewed the original reproductive and developmental toxicity study reports for prothioconazole.

Comment to 10.10.1 - 10.10.3 (Adverse effects on sexual function and fertility): I support the assessments and conclusions made in the CLH report. Effects on postnatal

development/fertility were only observed in the top dose group of 750 mg/kg in rats, a dose that is close to the limit dose of 1000 mg/kg for this study type and leads to extensive general toxicity. Therefore, the potential toxicological relevance of these findings is negligible. The results of this study therefore do not warrant any classification for fertility.

Comment to 10.10.4 - 10.10.6 (Adverse effects on development): I support the assessments and conclusions made in the CLH report. It can be concluded that there are two potentially critical points on prenatal development, namely increased rate of microphthalmia in the top group at 1000 mg/kg in rats, and increased rate of rudimentary supernumerary lumbar ribs in the top group at >= 750 mg/kg in rats. Supplementary data from mechanistic studies in rats are sufficient to mitigate these points. Along with a clear study in rabbits and applying a weight of evidence approach, I conclude that any classification for developmental toxicity higher than Category 2 would not be warranted.

Dossier Submitter's Response
Noted.
RAC's response
Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
25.05.2018	Germany	Bayer AG	Company-Manufacturer	6

Comment received

Comment to 10.10 (reproductive toxicity): Bayer supports and agrees with the assessments and conclusions made in the CLH report for prothioconazole including: the conclusion that no classification for adverse effects on sexual function and fertility; no classification for adverse effects on development of the offspring; and no classification for reproductive toxicity concerning effects on or via lactation is warranted. We also agree that the respective supporting data are conclusive.
As part of the advanced state of the art (quantum computational) scientific research being continuously undertaken by Bayer, we wish to include the latest findings which complement and further reinforce the existing body of scientific evidence explaining why prothioconazole does not cause the typical reproductive and developmental toxicity associated with several classical triazole fungicides:
• Following classical triazole treatments, two critical toxicological observations that may arise in rats are late foetal deaths and teratogenicity.
• The literature supports the conclusion that the underlying mechanisms are different, but both may involve inhibition of certain cytochrome P450 enzymes - specifically CYP19 (aromatase - essential for the conversion of androgens to oestrogens) and CYP26 (involved in catabolism of the endogenous morphogen, retinoic acid).
• It is assumed that CYP19 inhibition results in an insufficient oestrogen level to support pregnancy and that CYP26 inhibition may cause the observed teratogenicity (e.g. cleft palate and limb malformations).
• Our comprehensive data set shows that prothioconazole does not cause this reproductive and developmental toxicity associated with several classical triazole fungicides.
• Prothioconazole is chemically not a triazole, but a triazolinethione.
• It was, therefore, hypothesized that this structural chemical difference between prothioconazole and classical triazoles could lead to different ways of interaction with CYP19 and CYP26 enzymes. Indeed, publicly available scientific research confirms that

prothioconazole does not inhibit CYP19, reinforcing the hypothesis.
• The binding mode to the heme ferric ion in the active site of CYP450 enzymes has a strong influence on the inhibition of the enzymes. Publicly available UV spectroscopic data indicate that the interaction of prothioconazole with the heme ferric ion of CYP450 enzymes must be fundamentally different from the very typical interactions commonly observed for classical triazoles.
• To further elucidate the nature of electronic binding of prothioconazole to CYP450 enzymes Bayer recently generated respective quantum computational chemistry data. These data are presented for the first time in the separately submitted expert statement document and provide solid atomistic reasoning as to why prothioconazole exhibits a very weak and atypical binding mode to CYP450 enzymes, including CYP19 and CYP26, making it fundamentally different to that of classical triazoles.
The above data complement the already available Bayer proprietary animal and human metabolism data ("unique principle of rapid metabolic detoxification/excretion due to glucuronidation at the sulfur atom"), which are also summarized in this expert statement, and they complete the reasoning as to why prothioconazole does not cause the typical reproductive and developmental toxicity associated with several classical triazole fungicides, and also why prothioconazole has a generally very low overall toxicity in mammals.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment PTZ is a triazolinethione and does not cause the reprotox observed for several classical triazoles_sanitized.pdf

Dossier Submitter's Response

Thank you for these additional arguments to support the proposed positions on reproductive toxicity. These provide a computational chemistry basis for the conclusions that prothioconazole is not a triazole and, therefore, does not present the typical reproductive and developmental triazole toxicity.

RAC's response

Noted, thank you. RAC agrees that prothioconazole is not a triazole, but a triazolinethione, and that the pattern of reproductive and developmental toxicity is different from that of "classic" triazoles.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	Denmark		MemberState	7

Comment received

Increased incidence of microphthalmia was observed in a rat developmental study especially in the high dose (outside HCD) in the presence of maternal toxicity at 1000 mg/kg bw/day.
Prothioconazole did not induce microphthalmia in a rat strain less prone to developing this malformation (Wistar Hanover, Crl:WI(Han)). The maiximum dose tested in this strain was 750 mg/kg bw/day. It is difficult to know which rat strain is the more relevant to humans.

An inhalatory developmental toxicity study on Cyfluthrin was used to support the hypothesis that increased incidence of microphthalmia was a secondary non-specific effect of maternal toxicity.

The relevance of developmental toxicity study in Cyfluthrin is unclear because the compound caused hypoxia in the rats when administered via inhalation. Hypoxia during

development is known to be capable of inducing many types of malformations.

Observations supporting this:

• According to the submitter, the embryotoxic findings correlated with maternal toxicity (hypoxia with the resulting compensatory mechanisms of hypothermia and respiratory alkalosis, resulting in clinical signs of respiratory disturbances and hypoactivity).

• Oxygen supply (high-concentration group) resulted in reduction of maternal as well as developmental effects; in particular, the incidence of foetuses with microphthalmia was reduced. These observations support that hypoxia may be the primary MOA for development of microphthalmia/other malformations and maternal toxicity for Cyfluthrin in this study.

Since Cyfluthrin causes hypoxia (by inhalation) which may induce malformations, the effects observed in the cyfluthrin study cannot be used as evidence of a non-specific nature (via maternal toxicity) of the observed microphthalmia for prothioconazole.

Overall, it has not been sufficiently shown that the increased incidence of the malformation microphthalmia is a non-specific exacerbation of a spontaneously-occurring malformation in a specific strain resulting from maternal toxicity. Therefore the malformation is relevant for classification in category 2.

AGD was increased in the 2-generation study in both males and females and attributed to increased bw. How was data normalized to bw? It may be relevant for classification.

Dossier Submitter's Response

With regard to the rat strain used in the second rat developmental toxicity study, the Wistar Hanover, Crl:WI(Han), it is important to emphasise that this strain has a very low spontaneous incidence of microphthalmia. This strain is sensitive to specific effects on ocular development; the positive control substance retinoic acid (15 mg/kg bw) caused increased litter incidences of anophthalmia (41.7%), microphthalmia (16.7%), and small lens (8.3%).

In the cyfluthrin inhalation study, foetal development (reduced foetal and placental weight from the mid dose) was likely to have been retarded secondarily to the disturbance of maternal physiology (bradypnea, hypoxia, hypothermia, respiratory alkalosis) together with an increased incidence of the common spontaneous malformation microphthalmia in this rat strain (only at the high dose). The reduction in the microphthalmia incidences in the oxygen-enriched high dose group reflects the unspecific mode of action, considering the fact that also the correlating "unspecific" parameters placenta weights and foetus weights clearly improved in this group. In a personal communication, the applicant has referred to a publication that cites the following references on the effect of fever / hyperthermia on the incidence of anophthalmia and microphthalmia in humans (not summarised by the applicant nor reviewed by the dossier submitter):

Buys ML. Birth defects encyclopaedia. Dover, MA: Center for Birth Defects Information Services; 1990. Eye.

Knox EG, Lancashire RJ. London: HMSO; 1991. *Epidemiology of congenital malformations*.

Edwards MJ. Hyperthermia as a teratogen. Teratogenesis, carcinog, mutagen. 1986;6:563–582. [PubMed]

Jones KL. Philadelphia: WB Saunders; 1988. *Smith's recognizable patterns of human malformation*.

Spraggett K, Fraser FC. Teratogenicity of maternal fever in woman—a retrospective study. Teratology. 1982;25:78A.

Fraser FC, Skelton J. Possible teratogenicity of maternal fever. Lancet. 1978;ii:634. [PubMed]

Sulik KK, Cook CS, Webster WS. Teratogens and craniofacial malformations: relationships to cell death. Development. 1988;103(suppl):213–232. [PubMed]

The applicant considers hyperthermia to be a disturbance of maternal physiological equilibrium that is similar to the effects of the irritant substance cyfluthrin and the maternal dehydration resulting from prothioconazole-induced kidney failure.

The applicant has provided us with information to clarify that the AGD was normalized with the cube root of body weight (as suggested by Gallavan *et al*., 1999):

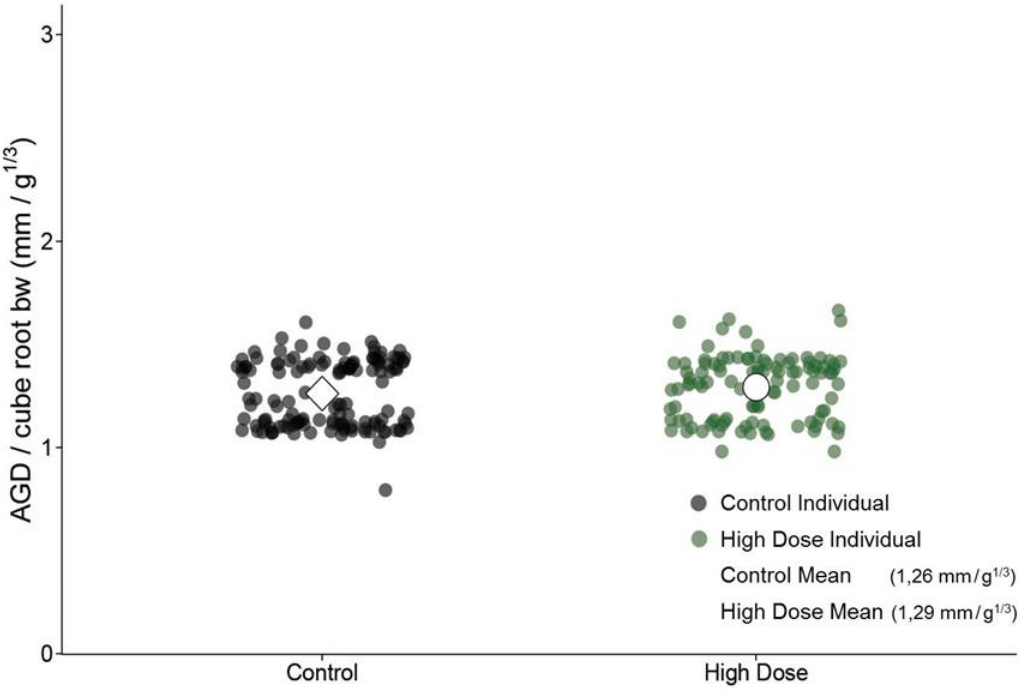

Figure 5.6.1/02- 11: Ratio AGD / cube root of body weight – female F2 pups at day of birth

[4] Gallavan, R.H., et al., 1999. Interpreting the toxicologic significance of alterations in anogenital distance: potential for confounding effects of progeny body weights. *Reproductive Toxicology*, **13**(1999):383-390.

RAC's response

Your opinion is noted, thank you. The cyfluthrin inhalation study will be discussed in the ODD. RAC agrees with the DS that the AGD-normalization has been performed according to what is recommended in the literature and that the outcome shown no differences when normalized for body weight.

Date	Country	Organisation	Type of Organisation	Comment number
11.05.2018	United Kingdom		Individual	8

Comment received

We each have more than 40 years of professional experience in the field of developmental and reproductive toxicity and have reviewed the original study reports and related documentation on prothioconazole developmental and reproductive toxicity as consultants for Bayer.

Regarding 10.10.1 through to 10.10.3 (Adverse effects on sexual function and fertility), we generally agree with the assessment of the toxicological profile and with the conclusion that no classification for adverse effects on sexual function and fertility is warranted.
Specifically, we consider that the small number of reproductive findings recorded at the high dose level of 750 mg/kg bwt/day, viz. slightly increased oestrous cycle length, marginally increased pre-coital interval, slight reduction in the number of implantation sites and minimally increased gestation length, were likely to be secondary to the severe maternal toxicity and should not be considered as an indication of any selective toxicity to reproduction. We consider that prothioconazole should not be classified as selectively toxic to reproduction.

Regarding 10.10.4 through to 10.10.6 (Adverse effects on development), we generally agree with the assessment of the toxicological profile and with the conclusion that no classification for adverse effects on development of the offspring is warranted.
Specifically, in view of the finding of microphthalmia in only one of the two gavage rat developmental toxicity studies and the absence of any eye malformations in the rat dermal developmental toxicity study, the rat two-generation study or in the rabbit gavage developmental toxicity study, we conclude that the observed microphthalmia does not indicate a teratogenic potential of prothioconazole. Microphthalmia only occurred in a strain of rat with a background of spontaneous microphthalmia, in the presence of marked maternal toxicity and at a dose level of 1000 mg/kg/day. There was no indication in any of the reproductive toxicity studies of the types of malformations more usually associated with other azole compounds, for example cleft palate and limb abnormalities.
The occurrence of rudimentary supernumerary ribs at the thoracolumbar border is a common variant in control rat fetuses. In developmental toxicity studies, an increase in the incidence of rudimentary supernumerary ribs is a frequent finding in treated groups at dose levels where maternal toxicity has been recorded, and it has been observed for a wide range of chemical entities. It is generally considered to be the result of a slight caudal shift in axial segmentation as a consequence of a non-specific maternal insult. In both of the prothioconazole rat gavage developmental toxicity studies clear maternal toxicity was recorded at the dose levels where an increased incidence of rudimentary supernumerary ribs was recorded and it may be concluded, therefore, that this finding did not represent a direct effect of prothioconazole upon rib development.
On a weight of evidence basis we conclude that prothioconazole should not be classified as a developmental toxin."

Dossier Submitter's Response

Noted, thank you.

RAC's response

Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
11.05.2018	United Kingdom		Individual	9

Comment received

I have worked in the areas of chemical risk assessment and developmental and reproductive toxicity for 45 years. As a consultant for the manufacturer, Bayer AG, I have assessed the original study reports and related documentation on prothioconazole reproductive toxicity.

With regard to adverse effects on sexual function and fertility (10.10.1 through to 10.10.3), I generally agree with the assessment of the toxicological profile and with the conclusion that no classification for adverse effects on sexual function and fertility is warranted. The effects observed on reproductive and developmental parameters were all small in magnitude, few achieved statistical significance and there was considerable inconsistency in the direction of the changes between generations. There was also no pattern of parental or offspring changes that was consistent with a plausible endocrine mode of action. The observed effects were most likely secondary to, and attributable to, the clear general systemic toxicity and effects on body weight gain. None of the effects observed, including the effects on parental oestrous cycles, had any impact on the overall reproductive capacity of the males or females. In my opinion, the results of this two-generation rat study do not warrant classification for reproductive toxicity.

With regard to adverse effects on development (10.10.4 through to 10.10.6), I generally agree with the assessment of the toxicological profile. Overall, the results of the developmental studies could trigger classification of prothioconazole for developmental toxicity with respect to the occurrence of microphthalmia in the rat.

Given that:

(1) there was considerable maternal toxicity at the dose of prothioconazole at which an increase in microphthalmia was observed;

(2) there was a greater degree of maternal toxicity in high-dose prothioconazole dams carrying fetuses with microphthalmia than in high-dose dams carrying unaffected fetuses;

(3) microphthalmia is a common spontaneous abnormality in the sub-strain of Wistar rat that showed an increase in microphthalmia at a high, maternally toxic dose of prothioconazole;

(4) a different Wistar sub-strain not prone to microphthalmia did not show any evidence of microphthalmia or other abnormalities at a maternally toxic dose of prothioconazole; and

(5) the rabbit did not show any increase in abnormalities at a maternally toxic dose of prothioconazole;

any classification for developmental toxicity higher than Category 2 would not be warranted.

Dossier Submitter's Response

Noted, thank you.

RAC's response

Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
15.06.2018	Austria		MemberState	10

Comment received

CLH report, chapter 10.10 reproductive toxicity:

Adverse effects on sexual function and fertility:

In a preliminary range-finding study (top dose 500 mg/kg bw/day) and a guideline-compliant two-generation reproduction study in Wistar rats (top dose 750 mg/kg bw/day), marked parental toxicity was observed in the top dose groups.
Offspring toxicity was restricted to the high dose group. Delayed preputial separation in the F1 pups correlated with retarded growth.
No specific effects on reproduction were observed, and non-classification for effects on sexual function and fertility is supported.

Adverse effects on development:

Several studies addressing the developmental toxicity of prothioconazole are available and summarized in the CLH-report:

1)Oral developmental toxicity study in Wistar rats (Hsd Cpb: WU) (1997) incl range finding study (1995):
Dose levels tested in main study: 0, 80, 500, 1000mg/kg bw/day

2)Oral developmental toxicity study in Wistar Hanover rats (Crl:WI(Han) incl range finding study(2004)
Dose levels tested in main study: 0, 20, 80, 750mg/kg bw/day

3)Dermal developmental toxicity study in Wistar Hannover rats (Crl: WI(Han), 2001
Dose levels tested: 62,5 and 250 mg/kg bw/day

4)Oral developmental toxicity study in Chinchilla rabbits (CHbb:CH, Hybrids) (1998), incl range finding study (1997)
Dose levels tested in main study: 0, 10, 30, 80, 350mg/kg bw/day

In both oral developmental studies in rats, the observed developmental effects were confined to the top dose groups which exhibited marked maternal toxicity.
In the first study (1997), an increase in microphthalmia was observed in the top dose group (above concurrent and available historical control data). It is evident from the presented data that this finding occurs spontaneously at relatively high incidences in this rat strain.
In the second oral developmental study in rats, a strain with virtually no background incidence for microphthalmia was selected, and the study design was adjusted to detect ocular effects. No microphthalmia was detected in this study.
In both oral developmental toxicity studies in rats, rudimentary supernumerary ribs (variation) were observed at dose levels causing maternal toxicity.
In the oral developmental toxicity study in rabbits, indications for developmental toxicity were evident at any dose level (top dose exhibited severe toxicity).

In conclusion – considering all available studies as well as the submitted additional

information and argumentation following the weight of evidence approach - the proposed non-classification of prothioconazole regarding reproductive toxicity is supported.
Dossier Submitter's Response
Noted, thank you for your support.
RAC's response
Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
15.06.2018	Germany	Bayer AG	Company-Manufacturer	11
Comment received				
With regard to the occurrence of rudimentary supernumerary 14th lumbar ribs in the developmental toxicity studies in rats (10.10.4.1, 10.10.5 and 10.10.6.) Bayer agrees with the assessment that the effect occurred only at very high doses that were severely toxic to the dams. Currently in the EU a "combined" NOAEL of 20 mg/kg bw/d for the formation of rudimentary 14th ribs in rats is set, considering the results of both the FIRST and the SECOND developmental toxicity studies in rats (EFSA PRAPeR 04 Meeting, September 2006). In the following a rationale is provided why this NOAEL should be set at at least 80 mg/kg bw/d, instead of 20 mg/kg bw/d. In the FIRST rat developmental toxicity study, dose-dependent increases in rudimentary 14th rib incidence were observed; however, as discussed on p.41 of the CLH report, the incidence seen in control animals was unusually low in this study (the second-lowest in 52 studies performed in that laboratory and rat strain). Therefore it can be concluded that no treatment-related increase of rudimentary supernumerary 14th ribs was observed at least at 80 mg/kg bw/d. This assessment is supported by the fact that in the SECOND rat developmental toxicity 80 mg/kg bw/d was a clear NOAEL for rudimentary supernumerary 14th ribs and the increase of this finding at the top dose of 750 mg/kg bw/d was only marginal. In the EFSA-DAR (2004, Prothioconazole – Volume 3, Annex B.6.: Toxicology - Prothioconazole) this NOAEL of 80 mg/kg bw/d in the SECOND study was stated to be "probably conservative". Meanwhile two benchmark dose (BMD) analyses of the rudimentary 14th ribs observed in the FIRST rat developmental toxicity study in rats were conducted, resulting in BMDL10 values (equivalent to NOAELs) of 318 mg/kg bw/d (according to EFSA 2016 guidance – see report M-579365-01-1) and 384 mg/kg bw/d (using US-EPA software – see report M-531958-01-1). Both reports are also submitted together with this public comment. A BMD approach provides quantitative support for selection of a NOAEL or reference dose via consideration of the shape of the dose-response curve using mathematical models. This methodology enables determination of a dose corresponding to a specified effect in a manner that is not limited to experimental doses and less dependent on dose spacing than the traditional NOAEL/LOAEL approach. Overall, it can be concluded that the true NOAEL for 14th rudimentary ribs is considerably higher than 80 mg/kg bw/d. In this context we would also like to refer to the published conclusion from Mylchreest and Harris (Mylchreest E, Harris SB (2013). Data interpretation: Using historical control data to understand supernumerary ribs, a common skeletal variation. In: Teratogenicity testing, methods and protocols, Barrow PC (editor), ISSN 1064-3745, ISBN 978-1-62703-130-1, Humana Press, Springer New York, Heidelberg, Dordrecht, London, 290-294) that, based on the prevalence of supernumerary rib in the population, marginal increases in the incidence of rudimentary 14th ribs may not have toxicological or biological significance				

and that rudimentary 14th ribs should not be considered biologically significant in the absence of more profound signs of developmental toxicity – which are clearly absent in the FIRST rat developmental toxicity study up to and including 500 mg/kg bw/d.
A NOAEL of at least 80 mg/kg for rudimentary 14th ribs was derived by the following Regulatory Bodies:
• PMRA Canada (Regulatory Note REG2007-03, Prothioconazole, 31 January 2007, p. 67)
• US-EPA (Prothioconazole: Human Health Risk Assessment, Jan. 23, 2007, p. 33; Pesticide Fact Sheet, Prothioconazole, March 14, 2007, p. 7)
• UK HSE as ECB-Rapporteur (REACH ANNEX XV, Proposal for Harmonised Classification and Labelling, March 2007, p. 13)
• FAO/WHO (Joint Meeting on Pesticide Residues, Report 2008, 193: 265, p. 271-272)
• EFSA PPR Panel (EFSA Journal 2009; 7(9):1167, Scientific Opinion on Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health, p. 117

ECHA note – An attachment was submitted with the comment above. Refer to public attachment BMD_public.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment BMD_confidential.zip

Dossier Submitter's Response

We note the comments on the NOAEL identified from the developmental toxicity studies in rats; the value will be considered during the EFSA peer-review process.

RAC's response

Noted, thank you. NOAEL-setting is not part of the basis for classification. The additational reports submitted will therefore not be included as part of the evaluation.

Date	Country	Organisation	Type of Organisation	Comment number
15.06.2018	Germany	Bayer AG	Company-Manufacturer	12

Comment received

With regard to the occurrence of microphthalmia in the FIRST developmental toxicity study in rats (10.10.4.1, 10.10.5 and 10.10.6.) we (Bayer) generally agree with the assessment.
As apparent from the historical control data, in the Wistar substrain Hsd Cpb:Wu microphthalmia occurs as a common spontaneous malformation and the slightly, not dose-related increased incidences at 80 and 500 mg/kg bw/d were clearly within the respective historical control range. At 1000 mg/kg bw/d very strong, sublethal maternal toxicity was present (see also previous comment on maternal toxicity (10.10.4.1)). By grouping the 1000 mg/kg bw/d maternal toxicity results separately for those dams that produced pups with microphthalmia and for those that did not have any pups with microphthalmia it became evident that the severity of maternal toxicity correlates positively with the degree of foetal toxicity (body weight decrease) and with the occurrence of microphthalmia. Therefore, we also consider the increased incidence of microphthalmia at the high dose level as an unspecific enhancement of the common spontaneous malformation microphthalmia secondary to disturbed maternal health in this certain Wistar substrain (Hsd Cpb:WU).

This assumption is further substantiated by an INHALATIVE developmental toxicity study in this same Wistar substrain in which exposure to another, sensory irritating compound caused, secondarily to reflectory induced maternal bradypnea / hypoxia / hypothermia /

respiratory alkalosis, a retarded foetal development with reduced foetal and placental weight together with an increased incidence of the common spontaneous malformation microphthalmia (at even higher foetal / litter incidences than in the prothioconazole study). In an ORAL developmental toxicity study, this test compound did not cause any microphthalmia at an approx. 10-fold higher systemic dose. Oxygen enrichment of the inhaled air partially compensated the bradypnea-related hypoxia and, thus, resulted in a reduction of the number of foetuses with microphthalmia at the same highest dose tested. The clear (but incomplete) improvement of the microphthalmia incidences reflects the asserted "unspecific and secondary to maternal toxicity" mode of action considering the fact that also the correlating "unspecific" parameters placenta weights and foetus weights clearly (but incompletely) improved. It is important to note that the enriched air oxygen content was just a proof of principle. The oxygen level in the air could not be increased to a level which could have eliminated maternal hypothermia, hypoxia and acid-base status because it would have become lung toxic. Based on the available data it can be concluded, that in Hsd Cpb:WU rats, a severely disturbed maternal physiological equilibrium (e.g., dehydration due to kidney toxicity or hypoxia due to sensory irritation) can enhance the occurrence of the common spontaneous malformation microphthalmia. In the rat strain used for the SECOND rat developmental toxicity study and for the two generation study, which has a "zero" spontaneous occurrence of this malformation (but which was validated for its sensitivity to specific oculo-teratogenicity with the positive control substance retinoic acid), the incidence of microphthalmia was not increased, even when tested up to doses that were severely toxic to the dams. The principle that maternal physiological disbalance (i.e., stress) can increase the incidence of common spontaneous malformation is well-known for mice: The background incidence of 1% for cleft palate (a common spontaneous malformation in mice) was increased to 69% by restraining the pregnant dams (Golub et al., Effects of restraint stress in gestation: Implications for rodent developmental toxicology studies, Birth Defects Research (Part B) 71: 26 - 36, 2004). The occurrence of distinct maternal toxicity also has an impact on warranty of classification for reproductive effects. According to the ECHA Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 5.0, July 2017, this constellation of maternal and reproductive / offspring effects does not warrant any reproductive toxicity classification. Specifically, the Guidance states: "Based on pragmatic observation, MATERNAL TOXICITY may, depending on severity, influence development VIA NON-SPECIFIC SECONDARY MECHANISMS, producing effects such as DEPRESSED FOETAL WEIGHT, RETARDED OSSIFICATION, and possibly resorptions and CERTAIN MALFORMATIONS IN SOME STRAINS OF CERTAIN SPECIES."
Dossier Submitter's Response
Noted, thank you for your comments.
RAC's response
Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
15.06.2018	Germany	Bayer AG	Company-Manufacturer	13

Comment received

With regard to maternal toxicity in the developmental toxicity studies in rats (10.10.4.1), we (Bayer) generally agree with the assessment.
In addition we would like to underline that in the context of results from the rat subacute and subchronic toxicity studies, maternal toxicity was primarily related to severe disturbances of the kidney function and systemic water and electrolyte homeostasis. This is a consistent finding in all toxicity studies in the rat and appears to be the characteristic toxicity of prothioconazole. In two rat 28-day studies strongly increased water intake was observed at 1000 mg/kg bw/d. In a 90-day study conducted in Wistar rats of the same substrain as used in the FIRST developmental toxicity study (Hsd Cpb:WU) at 500 mg/kg bw/d males and females exhibited strongly increased water intake, kidney damage and even a single case of death in one female in week 13 (possibly related to kidney failure). The renal “corticular tubular basophilia” observed in rat short term studies is assessed as a reactive regenerative response to a toxic insult. In the rat 1 year and oncogenicity studies, this lead to an increased incidence of "chronic progressive nephropathy" which even caused in the 1-year study an increased mortality at 750 mg/kg bw/d, and a high mortality rate in the 2-year study at 500-750 mg/kg bw/d.
A further indicator that a strongly maternally toxic dose had been reached in the FIRST developmental toxicity study at 1000 mg/kg is the clear effect on body weights which already started at 500 mg/kg.
In the Wistar rat substrain that was used in the SECOND developmental toxicity study (Wistar Hanover), dehydration and strongly increased water intake of pregnant dams was already observed at 500 mg/kg bw/d and caused, at 1000 mg/kg bw/d, 25 % mortality (pilot study). In the main study, at 750 mg/kg bw/d dehydration could not be fully compensated even by a drastically increased (up to >170 % of control) water consumption.
In the FIRST developmental toxicity study water intake was also drastically increased (up to 175 % of control) in the high dose, thus it can be concluded that the severity of maternal toxicity is comparable in BOTH developmental toxicity studies. The degree of maternal toxicity at the respective highest tested doses of both studies is considered very strong, even sublethal.

With regard to maternal toxicity in the two generation reproductive toxicity study in rats (10.10.2), we (Bayer) generally agree with the assessment.
In addition we would like to underline that, although water consumption had not been measured in this study, the respective effects determined in the SECOND developmental toxicity study / respective pilot study (strongly increased water intake and dehydration at >=500 mg/kg bw/d, mortality at 1000 mg/kg bw/d) are considered fully applicable also to the two generation study, because it was conducted in the same Wistar strain and also by gavage application. It can therefore be concluded that the degree of maternal toxicity at the high dose (750 mg/kg bw/d) of the two generation study was very strong, even sublethal. Please see also our respective comment to (10.10.4.1)

Dossier Submitter’s Response

Thnk you for your comments. We agree that severe maternal toxicity was induced at 1000 mg/kg bw/d in the high-dose groups of the developmental toxicity and two-generation reproduction studies.

RAC's response
Noted, thank you.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	14

Comment received

Adverse effects on sexual function and fertility:
In the 2-generation study, adverse effects on sexual function or fertility were observed at the highest dose level in both P and F1-generation: prolonged duration of gestation, decreased mean number of oestrus cycles and increased mean oestrus cycle duration, as well as increased mean time to insemination (particularly in the F1 generation), decreased number of corpora lutea in the P generation and decrease number of implantation sites and mean litter size in both generations.
The systemic toxicity at this dose level is not considered excessive in both sexes, especially in females (decreased bodyweight gains by max. 5%, decreased thymus weight (in P only), liver effects (increased weight, hepatocytomegaly in F1 only), kidney effects (multifocal cortical nephrosis in 4/30 and 6/30 P and F1 females respectively).

Considering the above adverse effects on sexual function and fertility and in the absence of marked parental toxicity, a classification for fertility should be considered.

Adverse effects on development :
Microphthalmia were observed in all treated groups in the first developmental toxicity study in rats (Anonymous, 1997a).
Considering only the historical control data obtained from studies conducted in a five-year period around the date of the study (see Annex I page 98-99) (i.e. 19 studies conducted from 1994 to 1998/study with prothioconazole conducted in 1996), the range of microphthalmia was 0-20% of litter affected (with 19 studies showing a range 0-7.1%, 1 study with a mean of 17.86% and 1 study with a mean of 20%). The mean of % litter affected was 4.5%.
Therefore, the incidences of microphthalmia observed after administration of prothioconazole were above the range of relevant HCD (0-20%) in the high dose group only (33.3%), but the incidences observed in the low (15.4%) and mid (13.6%) groups were far above the mean HCD (4.5%). Furthermore, statistical significance was noted in the low and high dose groups. The lack of a clear dose-relationship could be due to the slightly lower number of litters and foetuses available in the mid dose group.
As a conclusion, considering that microphthalmia were observed in all treated groups, in foetuses from several litters, and that the incidences, although not clearly dose-related (but could be due to a lower number of foetuses at the mid dose level) were far above the mean of relevant HCD at all dose levels, this malformation should be considered adverse and treatment-related from the low dose level onwards.

Rudimentary 14th ribs were observed in all treated groups in in the first developmental toxicity in rats (Anonymous, 1997a). The incidences of this finding were dose-related and statistically significant from the low dose level.
Considering only the historical control data obtained from studies conducted in a five-year period around the date of the study (see Annex II page 100-101) (i.e. studies conducted from 1994 to 1998/study with prothioconazole conducted in 1996), the maximum percent of litters affected was 32.0%.
Therefore, the incidences of rudimentary 14th ribs observed after administration of

prothioconazole were above the range of relevant HCD (max 32.0%) in all treated groups (42.3%, 54.5% and 62.5% in the low-, mid- and high-dose groups respectively).
As a conclusion, this variation should be considered adverse and treatment-related from the low dose level onwards. This variation was also observed in the dose-range finding study (Anonymous, 1995) and in the second developmental rat toxicity study (Anonymous, 2004b)

Conclusion on developmental toxicity:
An increased incidence of microphthalmia was observed in rats in several litters at all dose levels tested. In general, malformations are not considered to be secondary to maternal toxicity (see for example Fleeman et al. (2005)*) and no specific mode of action was investigated to conclude on a secondary non-specific consequence of other toxic effects. Furthermore, the occurrence of this malformation after prothioconazole administration is consistent with the toxicological profile of this class of chemicals often inducing craniofacial malformations.
Moreover, increased incidence of rudimentary 14th rib and presence of 5th caudal vertebral body as well as delayed ossification/development were observed in rats.
In rabbits, abortions, total litter resorptions, post-implantation losses were noted in the presence of maternal toxicity.
As a conclusion, based on the developmental effects observed in two species, including teratogenic effects in rats (specific malformations typical of the class of chemicals under consideration), a classification for adverse effects on development of the offspring should be considered for prothioconazole.

* Fleeman TL, Cappon GD, Chapin RE, and Hurtt ME. (2005) Effect of feed restriction during organogenesis on embryo-fœtal development in the rat. Birth Defects Research (Part B) 74:442-449.

Dossier Submitter's Response

Please see response to comment number 4.

Taking into account the toxicity exhibited in other studies at similar doses, we consider the maternal toxicity in the high-dose group of the two-generation study to have been severe. As noted in the CLH report, urine staining was likely to be a sign of effects on the kidney and water homeostasis systems, consistent with the findings of the repeated-dose toxicity studies. Histopathological changes in the kidneys were also observed at 750 mg/kg bw/d in the main study. It is therefore reasonable to surmise that the observed urine stains and dehydration were manifestations of kidney dysfunction; furthermore, cortical nephrosis was observed at this dose.

Support for this interpretation is provided by the developmental toxicity studies in rats. In the study with the same rat strain as was used in the two-generation study and at the same prothioconazole gavage dose of 750 mg/kg bw/d, water intake was drastically increased; a dose of 1000 mg/kg bw/d in the supportive range-finding study resulted in the deaths, from dehydration, of 25% of dams. Indications of maternal toxicity in these developmental toxicity studies (range-finding and main) are considered to be applicable to the two-generation study (same doses, strain and gavage administration).

The dossier submitter therefore considers that 750 mg/kg bw/d was a clearly maternally-toxic dose.

Reproductive performance was not affected at any dose level: prothioconazole administration had no effect on mating, fertility or gestation indices. Slight changes in some parameters (number of oestrus cycles, implantation sites, litter size, time to insemination, duration of gestation), often without statistical significance and within historical control ranges), only occurred at 750 mg/kg bw/d and are not regarded by the RMS to be specific indications of effects on reproduction; rather, they were most likely to be either not treatment-related effects or secondary to the maternal toxicity. In a personal communication, the applicant has cited publications that report an effect of kidney failure upon oestrus cyclicity:

da Costa e Silva A, Campos KN, Souza PF, Albuquerque RH, Martinelli JG, Campos GP, et al. Effect of experimental uremia upon the estrous cycle in rats. Nephron. 1987;47(1):70-2.

Krieg RJ, Tokieda K, Chan JC, Veldhuis JD. Impact of uremia on female reproductive cyclicity, ovulation, and luteinizing hormone in the rat. Kidney international. 2000;58(2):569-74.

Regarding the incidence of microphthalmia in rats, only at 1000 mg/kg bw/d was the level above the relevant historical control data. The CLH report describes the inter-group variability that can occur in the incidence of microphthalmia in this rat strain. In particular, in one study conducted the year before the prothioconazole study and another conducted the year after, the control incidence was higher than in the low- and mid-dose prothioconazole groups. Therefore it cannot be concluded that the cases of microphthalmia at 80 and 500 mg/kg bw/d in the first rat developmental toxicity study were a result of prothioconazole administration. No cases were recorded in the second study, which used a rat strain with a very low spontaneous incidence of microphthalmia; in this study, no cases of microphthalmia were recorded in any dose group, even at a very high dose of prothioconazole.

The dossier submitter does not agree that an increased incidence of supernumerary 14th ribs supports a classification for developmental toxicity. As explained in the CLH report, supernumerary rib is a very common skeletal variation in the presence of maternal toxicity and may even be used in itself as an indicator of maternally-toxic doses. The historical control data also show the inherent variability and spontaneous occurrence of this variation. With regard to the 5th caudal vertebral body, this refers to the incidence of 5th caudal vertebral bodies that are not yet ossified; i.e., it is a slight retardation in ossification that reflects retarded development.

The maternal toxicity in rabbits was very severe, including deaths, substantially reduced food consumption and body-weight loss. It is generally recognised that rabbits are susceptible to abortions, litter resorptions and post-implantation losses under such conditions of severe maternal toxicity. These findings do not indicate a specific effect of prothioconazole on development.

The dossier submitter notes that prothioconazole is not a triazole. In these studies it did not induce the typical toxicological profile of the triazole class; i.e., craniofacial malformations, predominantly cleft palate.

RAC's response

Your opinion is noted, thank you. RAC acknowledges that this is a case between no classification and classification in category 2, for both sexual function and fertility and development, that will be discussed in the ODD. With regard to maternal toxicity, RAC agrees that although maternal toxicity occurred, it is regarded as not being excessive.

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	Spain		MemberState	15

Comment received

Reproductive toxicity (Spanish Ministry of Health Comments)

The Spanish CA considers that category 2 for development (H361d) could be proposed according to the effects observed in a rat developmental study (1997a). This proposal of classification is based on the presence in this study of microphthalmia (including two cases of bilateral microphthalmia) out of historical control data (HCD) for both fetuses and litters at the highest tested dose level of 1000 mg/kg bw/d. This malformation, even in presence of maternal toxicity, is regarded relevant for development. The following considerations on this lesion have been taken into account in our classification proposal.
Microphthalmia was observed at 1000 mg/kg bw/d in presence of maternal toxicity manifested by significant decreases of bodyweight gain, net gain and food consumption, increase of water consumption and also severe kidney effects. It has to be noted that this dose level seems to be high for this teratogenicity study (14-days of administration; GD 6-19) regarding maternal toxicity also seen at 500 mg/kg bw/d. Besides, the malformation was not observed in other strain of rat or in rabbit and the fetuses with microphthalmia exhibited a poorer condition than those without the lesion. The dossier submitter concluded that microphthalmia can be a direct consequence of the poor condition of parental animals.
Although the Spanish CA agrees with the considerations on the high tested dose level, more doubts arise with the possible relation between microphthalmia and maternal toxicity. There are cases in which a relation between a lesion and parental toxicity can be established. For instance, the increased incidence of rudimentary 14th ribs seen at 1000 mg/kg bw/d in this study can be associated to maternal toxicity since effects in fetuses such as skeletal variations can be related to the reduction of bodyweight and food consumption in parental animals. However, there are no data indicating that maternal effects observed in the rat developmental study (1997a) with prothioconazole can cause microphthalmia. There is an example included in the CLH report showing an increased incidence of microphthalmia in rats treated with cyfluthrin after inhalation of the test substance. However, the pattern of maternal toxicity after inhalation treatment with cyfluthrin (hypoxia resulting in clinical signs of respiratory disturbances and hypoactivity) is different than the observed after prothioconazole treatment. Consequently, microphthalmia cannot be univocally considered a secondary consequence of maternal toxicity and category 2 (H361d) is proposed according to CLP criteria.
Additionally, the Spanish CA wants to note some information pointing out the possible influence of 1,2,4 triazole in the occurrence of microphthalmia. This compound was identified as a metabolite (M13) in rat urine (≈2%) according to data available in the EFSA Public Consultation on the active substance prothioconazole (Volume 3-B.6; ADME). Furthermore, the Spanish CA, in the context of regulatory purposes for other triazole fungicides, has evaluated a teratogenicity study in rats performed with 1,2,4 triazole in which 2 cases of microphthalmia and another case of anophthalmia were observed from a total number of 228 fetuses examined at 100 mg/kg bw/d (highest tested dose level). HCD available in the study report for the same strain of rat in 8 studies showed incidence of microphthalmia in 6/1344 fetuses (no range available). These HCD support a higher incidence for this lesion indicating a possible correlation between the lesion and 1,2,4 triazole treatment. Currently, a CLH Report for 1,2,4 triazole is under public consultation including data from the mentioned study report (reference: Renhof, 1989b).

Consequently, taking into account all the available information, classification as Repr.2 (H361d) is proposed by the Spanish CA for prothioconazole.

Dossier Submitter's Response

Please see response to comment number 4.

The applicant has provided the following information in response to the comment on the occurrence of microphthalmia following 1,2,4-triazole administration:

Here are the data from the Renhof study (Renhof, 1988a, Report no. 17401, experimental phase Feb-March 1985):

1,2,4-triazole T5019339

Group	Animal no.	No. of malformed foetuses	Type of malformation
Control	575	1	microphthalmia, bilateral
10 mg/kg	561	1	microphthalmia, right side
30 mg/kg	550	1	false posture of right hind leg
100 mg/kg	535	1	microphthalmia, left side
	573	1	dysplasia and assymetry of body of vertebrae and vertebral arches of thoracic spine and abnormal position of one rib
	576	1	anophthalmia
	587	1	microphthalmia, right side

Each of the malformations in the 100 mg/kg group affected only one foetus. They were predominantly eye deformities which were also observed in the control group. From their type and frequency they are known to be spontaneous malformations (see page 27 in the appendix).

It is evident from that study that also the concurrent control shows one case of microphthalmia (even bilateral). The rat strain in this study was: Bor: WISW (SPF Cpb).

A follow-up study was conducted in the same rat strain and laboratory with even a higher dose tested (200 mg/kg – Renhof, 1988b, Report No. 17402, experimental phase October 1986 to August 1987). Here are the results from that study:

Group	no. of dam	no. of foetus	type of malformation
Control	2062	5	multiple malformation
	2070	66	hydronephrosis
		70	undescended testicle
	2124	499	microphthalmia, left side
		501	microphthalmia, left side
	2130	562	undescended testicle
100 mg/kg	2097	280	undescended testicle
	2114	403	undescended testicle
	2119	457	undescended testicle
		463	undescended testicle
	2120	465	undescended testicle
		472	undescended testicle
		471	hydronephrosis, left side
	2122	479	undescended testicle
		481	undescended testicle
	2131	572	undescended testicle
		574	undescended testicle
	2133	593	undescended testicle

200 mg/kg	2065	55	cleft palate
	2068	59	undescended testicle
		58	humeral dysplasia
	2071	119	hydronephrosis
	2077	124	general oedema, false posture of hind legs
	2082	173	hydronephrosis, undescended testicle
		175	undescended testicle
	2086	210	hydronephrosis
	2092	238	long bone dysplasia
	2096	253	cleft palate
		254	hydronephrosis
	2098	300	undescended testicle
	2099	307	undescended testicle
		308	hydronephrosis
		310	hydronephrosis
	2100	311	undescended testicle
	2109	383	hydronephrosis
	2110	390	long bone dysplasia, cleft palate
		392	cleft palate
	2113	408	diaphragmatic hernia

The appearance of cleft palates and malformations of the hind legs in the 200 mg/kg group in particular point to 1,2,4-triazole having a teratogenic effect (see historic control data in appendix, page 237).

It is evident from that study that two control foetuses from one litter had microphthalmia but no foetuses at 100 mg/kg and 200 mg/kg. Based on these data, a treatment-related effect of 1,2,4 triazole on microphthalmia in both Renhof studies is highly unlikely.

Furthermore, here are additional historical control data for the rat strain and laboratory:

Historical Data of Control Groups (1983-1984) - **Spontaneous Malformations**

Species: Rat Strain: BOR:WISW (SPF Cpb)

Year	Study	Number of examined litters	Litters with malformations number	%	Number of examined fetuses	Fetuses with malformations number	%	Type of Malformation
1983	T5007693++	22	1	4.55	210	2	0.95	rib eminences
1983	T6007810++	22	1	4.55	218	1	0.46	cryptorchism
			1	4.55		1	0.46	microphthalmia, anophthalmia
1983	T7007811	24	0		245	0		
1983	T6008026	25	0		259	0		
1983	T5008025	24	1	4.17	247	1	0.40	dropsy, tubular bone dysplasia, rib eminences, sigmoid spine
1983	T2008392	12#	0		120	0		
1983	T2008626	12	1	8.33	114	1	0.88	microphthalmia
			1	8.33		1	0.88	general edema
1983	T0008714	22	1	4.55	207	1	0.48	hydronephrosis
			2	9.09		2	0.97	tubular bone dysplasia
1983	T1008788	22	0		214	0		
1984	T6008774	19	0		198	0		
1984	T5016710	24	1	4.17	254	1	0.39	tubular bone dysplasia
			1	4.17		1	0.39	microphthalmia
1984	T9016877	22	1	4.55	173	1	0.58	spinal kink
			1	4.55		1	0.58	microphthalmia
			1	4.55		1	0.58	internal hydrocephalus
1984	T3016961	9#	0		77	0		
1984	T9017029	18	0		165	0		
1984	T4017646	6	0		70	0		
1984	T7018512	21	0		202	0		
1984	T8019035	22	4	18.18	205	4	1.95	microphthalmia
			1	4.55		1	0.49	tubular bone dysplasia
1984	T8017569++	8	0		87	0		

++ intravenous application # including 1 animal with total resorption

It can be seen that microphthalmia and anophthalmia are common spontaneous malformations in that rat strain in 1983 and 1984 which cover also the respective incidences at 100 mg/kg in the Renhof (1988a) study.

Historical Data of Control Groups (1985-1986) – Spontaneous Malformations

Species: Rat Strain: BOR:WISW (SPF Cpb)

Year	Study	Number of examined litters	Litters with malformations number	%	Number of examined fetuses	Fetuses with malformations number	%	Type of Malformation
1985	T0019190	17	1	5.88	199	1	0.50	cryptorchism
1985	T5019339	21	1	4.76	231	1	0.43	microphthalmia
1985	T3019472	20	2	10.00	181	2	1.10	hydronephrosis
			1	5.00		1	0.55	hydroureter
1985	T9019676	8	0		80	0		
1985	T6019475	10#	1	11.11	89	1	1.12	agnesia of the caudal spine, pelvic constriction, fusions in sacral zone
1985	T6019637	8	0		91	0		
1985	T5019825	16##	1	8.33	122	1	0.82	microphthalmia
1985	T8019891	16	0		134	0		
1985	T9019937	9	0		90	0		
1985	T4019824	6#	1	20.00	46	1	2.17	exencephaly
1985	T5019960	10	0		92	0		
1985	T2020361	20	0		222	0		
1985	T5020977	14#	0		131	0		
1985	T0020125+++	25	1	4.00	271	1	0.37	microphthalmia
1985	T3020650	20#	1	5.26	194	1	0.52	anophthalmia
1985	T7020023	9	1	11.11	84	1	1.19	tubular bone dysplasia
1986	T3021686+++	23	1	4.35	206	1	0.49	anophthalmia
1986	T9021899	23#	1	4.55	219	2	0.91	degeneration in cerebral hemisphere
1986	T5022506	24	1	4.17	232	1	0.43	microphthalmia
			2	8.33		2	0.86	tubular bone dysplasia
1986	T5023280	8	0		72	0		
1986	T1023484	23	2	8.70	223	2	0.90	microphthalmia
			1	4.35		1	0.45	omphalocele
			1	4.35		1	0.45	general edema, dysplasia of the limbs
1986	T8023869	24	2	8.33	284	2	0.70	hydronephrosis
			2	8.33		2	0.70	humeral dysplasia
			1	4.17		1	0.35	general edema, dysplasia of the limbs
1986	T3024250	21	1	4.76	253	2	0.79	microphthalmia
			2	9.52		2	0.79	cryptorchism
			1	4.76		1	0.40	hydronephrosis
			1	4.76		1	0.40	multiple malformation

+++ inhalation

including 1 animal with total resorption
including 4 animals with total resorption

It can be seen that microphthalmia and anophthalmia are common spontaneous malformations in that rat strain in 1985 and 1986 which cover also the respective incidences at 100 mg/kg in the Renhof (1988a) study.

Historical Data of Control Groups (1987)

Spontaneous Malformations

Species: Rat — Strain: Hsd Cpb:WU

Year	Study	Number of examined litters	Litters with malformations no.	%	Number of examined fetuses	Fetuses with malformations no.	%	Type of Malformation
1987	T1024591	10	0		112	0		
1987	T6026017	10	1	10.00	118	1	0.85	cryptorchism
			1	10.00		2	1.69	tubular bone dysplasia
1987	T6025171+	24	1	4.17	230	1	0.43	hydronephrosis
			2	8.33		2	0.87	microphthalmia
			1	4.17		1	0.43	dysplasia of the limb bones
			1	4.17		1	0.43	cleft palate, edema, closed gastroschisis
1987	T7025604	21	1	4.76	220	2	0.91	humeral dysplasia
1987	T6023777	21	1	4.76	232	1	0.43	cryptorchism
			1	4.76		1	0.43	microphthalmia
			2	9.52		5	2.16	dysplasia of the limb bones
			1	4.76		1	0.43	general edema
1987	T1027435	20	1	5.00	185	2	1.08	hydronephrosis
			2	10.00		2	1.08	microphthalmia
			1	5.00		1	0.54	anophthalmia
1987	T5027196	22	1	4.55	193	1	0.52	renal pelvis dilation
			1	4.55		1	0.52	dysplasia of the limb bones, kinked tail

Percentage of fetal skeletal/visceral malformations refers to total number of fetuses.

+ dermal application

Strain Hsd Cpb:WU is identical with BOR:WISW (SPF Cpb)

It can be seen that microphthalmia and anophthalmia are common spontaneous malformations in that rat strain in 1987 which cover also the respective incidences at 100 mg/kg in the Renhof (1988a) study.

Historical Data of Control Groups (1988)

Spontaneous Malformations

Species: Rat — Strain: Hsd Cpb:WU

Year	Study	Number of examined litters	Litters with malformations no.	%	Number of examined fetuses	Fetuses with malformations no.	%	Type of Malformation
1988	T1027679	9	0		76	0		
1988	T2029650	21	2	10.00	200	3	1.50	dysplasia of the limbs
			1	5.00		1	0.50	microphthalmia
			1	5.00		1	0.50	thin tail
1988	T1029424	24	2	8.33	211	2	0.95	microphthalmia
			1	4.17		1	0.47	kinked tail, micrognathia
			2	8.33		2	0.95	dysplasia of the limb bones
			1	4.17		1	0.47	dilation of brain ventricles
			1	4.17		1	0.47	thin tail
1988	T0030007	18	1	5.56	187	1	0.53	cryptorchism
1988	T8030221--	21	0		212	0		
1988	T0030368	22	2	9.52	209	3	1.44	microphthalmia
			1	4.76		1	0.48	cryptorchism
1988	T7030455	8	1	12.50	86	1	1.16	cryptorchism
			1	12.50		1	1.16	diaphragmatic hernia

Percentage of fetal skeletal/visceral malformations refers to total number of fetuses.

-- examination not complete

Strain Hsd Cpb:WU is identical with BOR:WISW (SPF Cpb)

It can be seen that microphthalmia is a common spontaneous malformation in that rat strain in 1988 which covers also the respective incidence at 100 mg/kg in the Renhof (1988a) study.

It is therefore concluded that 1,2,4 triazole did not cause microphthalmia and anophthalmia in the Renof 1988a and b studies. It is furthermore concluded that the fact that 1,2,4 triazole is a minor systemic metabolite of prothioconazole in rats has no relevance in the assessment of the microphthalmia observed in the first prothioconazole rat developmental toxicity study.

RAC's response

Your opinion is noted, thank you. RAC acknowledges that this is a case between no classification and classification in category 2, for both sexual function and fertility and development, that will be discussed in the ODD. RAC agrees with the DS that the basis for 1,2,4-triazole causing microphthalmia is weak, and together with being a minor systemic metabolite to prothioconazole, it likely does not have an influence on the incidence of microphthalmia observed following prothioconazole exposure.

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	Germany		MemberState	16

Comment received

A classification in Repr. 2, H361d may be justified.
The DE-CA has repeatedly evaluated the mammalian toxicology of prothioconazole in the EU pesticide framework. In 2007, EFSA has published its "Conclusion regarding the peer review of the pesticide risk assessment of the active substance prothioconazole" (EFSA Scientific Report (2007) 106, 1-98, Conclusion on the peer review of prothioconazole). Both considered that classification as Repr. 2, H361d (Suspected of damaging the unborn child) would be appropriate. Since then, no new data has been presented that would affect this assessment.

Detailed justification:
1. Our evaluation of the study by Anonymous (1997a) is in agreement with the evaluation by the DS in that the dose of 1000 mg/kg bw /d is a clear effect level for microphthalmia in this strain of rats. However, we are not totally convinced that the increases in prevalence vs. the control group at lower doses (80 and 500 mg/kg bw/d) are not substance-related. Microphthalmia observations in both groups were in the upper quartile of the overall control range and definitely above the rather low control range in the year the study was conducted. Unfortunately, the data are insufficient for a definite conclusion for the following reason:
The only standard method of foetal examination which reliably detects microphthalmia is soft tissue evaluation and this was conducted only for 50 % of rat foetuses in the study by Anonymous (1997a), in concordance with guidelines. Overall, only 60 % of the microphthalmia cases were noticed at external inspection of the foetuses. The rest (between 15 % and 100 % in the different dose groups) was identified only by soft tissue examination. Therefore, the standard foetal examination cannot establish the true prevalence of microphthalmia in a study. Moreover, the ability of the foetal examination procedure to detect this malformation must be considered inadequate in study groups where the prevalence rate is low (as in controls and low dose groups). Findings under these conditions are mostly by chance and not by design.

2. The study by Anonymous (2004b) conducted in a different rat strain with a zero-background incidence of microphthalmia did not demonstrate a substance-related increase in this malformation at any dose up to 750 mg/kg bw/d. The foetal examination in this study is considered totally adequate. The heads of all foetuses were examined for eye malformations and eyes were weighed as well so that no case of microphthalmia would have been missed. The NOAEL for microphthalmia in this study and this strain therefore is 750 mg/kg bw/d. It must be considered, however, that in a multigeneration study conducted earlier in the same strain Anonymous (2001e) at this dose a single pup with a small eye was seen. As the strain is stated not to exhibit this type of malformation in control groups, the finding must be regarded as substance-related, resulting in a NOAEL of 100 mg/kg bw/d.

3. In an oral developmental toxicity study on prothioconazole in rats microphthalmia was found in the foetuses of the highest dose group. The incidence of this malformation was outside the historical control data. There is no experimental evidence available that systemic toxicity in the dams (i.E. maternal toxicity) may be causal for microphthalmia. Even if the malformation occurs spontaneously increased in this rat strain, it is regarded as a decisive effect, which can justify a classification in Repr. 2, H361d.
In developmental toxicity studies with other triazoles, such as penconazole and

propiconazole, the malformation microphthalmia was also found in rat foetuses.

4. Prothioconazole-desthio has been identified as being teratogenic given orally in two species (rat, rabbit) and dermally in one (rat). Although the plasma levels of this metabolite seem to be only 2-4 % of prothioconazole plasma concentrations, no data on placental transfer and embryo exposure are available. The conclusion of the DS that prothioconazole-desthio has no impact on the results of prothioconazole developmental toxicity studies therefore cannot be supported. Moreover, the amount of metabolite generated by other species, including humans, is unknown.

In case of a classification potency considerations should also be considered. According to the appraisement of the DE-CA the general concentration limit value for a classification as category 2 corresponds to the potency considerations for this substance.

Dossier Submitter's Response

Please see response to comment number 4.

We note that the expert committee for providing opinions on classification and labelling within the EU is RAC, not EFSA's meetings.

Additionally, new data have become available since the EFSA conclusion was published in 2007.

This includes comparative *in vitro* metabolism data, which shows that the amount of prothioconazole-desthio formed in human hepatocytes is comparable with that formed in rat hepatocytes. Only trace amounts of prothioconazole-desthio were formed systemically in rats (maximum 0.5% of the administered dose), and, supported by the *in vitro* comparative metabolism data, it can be postulated that only trace amounts would be formed in humans. It should also be noted that prothioconazole-desthio caused the 'typical' triazole malformation cleft palate, but not microphthalmia; therefore it seems unlikely that prothioconazole-desthio contributed to the non-specific occurrence of microphthalmia. Cleft palate did not occur in the prothioconazole studies.

Also, the sensitivity of the rat strain used in the second, supplementary developmental toxicity study (with a very low spontaneous incidence) to specific ocular teratogenicity was demonstrated with the positive control substance retinoic acid.

Additional information has been submitted during this public consultation, namely, quantam chemical calculations that show that prothioconazole is not a triazole (see comment 6). Therefore, it does not seem appropriate to compare the toxicology of prothioconazole with the developmental toxicity of triazole chemicals. Furthermore, in a personal communication to the dossier submitter the applicant has provided information that questions the occurrence of microphthalmia in rats administered penconazole (DAR, 2007 and EFSA conclusion, 2008) and propiconazole (CLH report, 2015).

With regards to the comment about a case of a small eye in the rat strain with a very low spontaneous background incidence of microphthalmia, the applicant has provided us with the following information and analysis:

The supplementary rat developmental toxicity study with prothioconazole (Anonymous, 2004b) was conducted in February / March 2004 in Wistar Hanover (Crl:WI(HAN)) rats.

This rat strain was called a "zero historical control background incidence" strain for microphthalmia in the study report, based on the following respective historical control data for Microphthalmia and Anophthalmia (in-life March 1998 to January 2002):

Historical Control for the
Incidence of Microphthalmia/Anophthalmia[a]
in the Wistar Hanover Rat [Crl:WI(HAN)]

Laboratory I.D. No.	In-Life Exposure	Breeder	Diet	Housing	Conducting Laboratory	Unit	Microphthalmia	Anophthalmia
01-T12-EW	07/01-01/02 (GD 6-19)	Charles River Raleigh, NC	Purina Mills Rodent Lab Chow 5002 Meal	Separately in suspended polycarbonate cages	Bayer CropScience LP Toxicology Stilwell, KS	Litter - Fetal -	0/26 (0%) 0/143 (0%)	0/26 (0%) 0/143 (0%)
99-T12-CL	06/99-12/99 (GD 6-17)	Charles River Raleigh, NC	Purina Mills Rodent Lab Chow 5002 Meal	Separately in suspended polycarbonate cages	Bayer CropScience LP Toxicology Stilwell, KS	Litter - Fetal -	0/26 (0%) 0/124 (0%)	0/26 (0%) 0/124 (0%)
98-622-QZ (JAU 6476)	08/98-12/98 (GD 0-19)	Charles River Raleigh, NC Chow 5001-4	Purina Mills Rodent Lab polycarbonate cages	Separately in suspended Stilwell, KS	Bayer CropScience LP Toxicology	Litter - Fetal -	0/23 (0%) 0/106 (0%)	0/23 (0%) 0/106 (0%)
98-612-PL	03/98-9/98 (GD 0-19)	Charles River Raleigh, NC	Purina Mills Rodent Lab Chow 5001-4	Separately in suspended polycarbonate cages	Bayer CropScience LP Toxicology Stilwell, KS	Litter - Fetal -	0/22 (0%) 0/98 (0%)	0/22 (0%) 0/98 (0%)

The multigeneration study (Anonymus 2001e) with prothioconazole was also conducted in Crl:WI(HAN) rats and had an in-life phase of February – November 1999. For this earlier time point further historical control data are available for the same rat strain and test laboratory which include one case of anophthalmia in the time range 1994-1997 (this time range is considered relevant for the multigeneration study:

Bayer Corporation 98-612-PL

HISTORICAL CONTROL DATA (1994 - 1997)

FETAL VISCERAL OBSERVATIONS (continued)

	FETUSES N	%	RANGE OF MEANS %	%	LITTERS N	%	RANGE OF MEANS %	%
NUMBER EVALUATED	1481				247			
Live	1480							
Dead	1							
URINARY: V KIDNEY, DILATED PELVIS (PELVES)	6	0.4	0.0	1.2	6	2.4	0.0	6.9
URINARY: M KIDNEY, HYPOPLASIA	3	0.2	0.0	1.4	2	0.8	0.0	3.8
URINARY: M KIDNEY, MALPOSITIONED	1	0.1	0.0	0.7	1	0.4	0.0	4.2
WILSON TECHNIQUE: M BRAIN, DILATED VENTRICLES	4	0.3	0.0	2.2	3	1.2	0.0	11.1
WILSON TECHNIQUE: M ANOPHTHALMIA	1	0.1	0.0	0.7	1	0.4	0.0	4.2
WILSON TECHNIQUE: M RETINAL FOLDING	2	0.1	0.0	1.1	1	0.4	0.0	3.7
TOTAL FETAL VISCERAL OBSERVATIONS[1]	130	8.8			88	35.6		

M = Malformation; V = Variation; I = Incidental
[1] Total includes the number of fetuses/litters that exhibited at least one visceral finding.

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The observation of one pup with unilateral "small eye" in the multigeneration study (Anonymus, 2001e), was a transient finding during clinical observations. It was observed in pup #3 from dam VH3106 only during days 30 to 37 (18-Jun-99 to 25-Jun-99). From the day of birth (19-May-99) until day 29 (17-Jun-99) all pups were normal. On Day 42 (30-Jun-99) for all pups "no remarkable clinical observations" was noted:

A TWO GENERATION REPRODUCTIVE TOXICITY STUDY WITH
JAU 6476 IN THE WISTAR RAT
P ADULTS/ F1 PUPS
INDIVIDUAL CLINICAL OBSERVATIONS -- CHRONOLOGICAL LISTING FEMALES

LEVEL III

ANIMAL#	CATEGORY	DATE	GRADE	OBSERVATIONS	
VH3106	Normal	19-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	20-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	21-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	22-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	23-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	24-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	25-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	26-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	27-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	28-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	29-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	30-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	31-MAY-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	1-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	2-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	3-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	4-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	5-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	6-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	7-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	8-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	9-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	10-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	11-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	12-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	13-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	14-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	15-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	16-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Normal	17-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Pups	18-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	18-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Normal	19-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	19-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	20-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	20-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	21-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	21-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	22-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	22-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	23-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	23-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	24-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	24-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Normal	25-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	3 PUPS
	Pups	25-JUN-99	P	SMALL EYE RIGHT	PUP# 3
	Pups	29-JUN-99	P	SALIVATION PRIOR TO DOSING.	PUP# 12
	Normal	30-JUN-99	P	NO REMARKABLE CLINICAL OBSERVATIONS	ALL PUPS
	Pups	4-JUL-99	P	SALIVATION PRIOR TO DOSING.	PUP# 12
	Pups	20-JUL-99	P	SALIVATION PRIOR TO DOSING.	PUP# 6
	Pups	21-JUN-99	P	SALIVATION PRIOR TO DOSING.	PUP# 6
	Pups	22-JUN-99	P	SALIVATION PRIOR TO DOSING.	PUP# 6

Microphthalmia is manifested during gestation and can be observed even at birth or before eye opening (day 15 – 17) via external examination (usually described as "no eye bulge" protruding underneath the closed eyelids). Clear cases of microphthalmia should be visible in detailed clinical observations also in foetuses before weaning at day 21 and thereafter. Considering the fact that the small eye in pup #3 was observed from day 30 – 37, but not before or after, and assuming that no respective errors occurred during the clinical observations, it is concluded that the observed finding is no case of the foetal malformation "microphthalmia", which would have been present even before birth and would not have been reversible. But even if the transiently observed "small eye" would have been a true microphthalmia, it is considered highly unlikely that it would represent a treatment-related effect of prothioconazole for the following reasons:

- *Transient unilateral "small eye" occurred at 750 mg/kg bw/d (gavage) only in one of 280 F1 pups and in none of 208 F2 pups*
- *The objective eye measures (weight and morphometry) applied in the supplementary rat developmental toxicity (Anonymous, 2004b) showed now indication for smaller eyes at 750 mg/kg bw/d (gavage) in 241 fetuses*
- *The historical control data (1994 – 1997) show that rare cases of spontaneous eye malformations can occur in that rat strain*

- *The suspicion that the finding "small eye" might be caused by prothioconazole due to an assumed common triazole class effect is not justified since the aforementioned (point 2.) new quantum chemical calculations show that prothioconazole is chemically and toxicologically not a triazole*
- *But even if a triazole class effect (via CYP26 inhibition and resulting imbalance of retinoic acid equilibrium) would be assumed for prothioconazole, the validation of the Crl:WI(HAN) rat strain for its sensitivity to specific oculo-teratogenicity with the aforementioned (point 3.) positive control substance retinoic acid should be considered. This validation study showed that cleft palate is the dominant effect of retinoic acid (fetal (litter) incidences: 72% (86%)), followed by lower incidences of anophthalmia (fetal (litter) incidences: 22% (42%)) and even lower incidences of microphthalmia (fetal (litter) incidences: 6% (17%)). So if prothioconazole would act as a classical triazole via CYP26 inhibition, one would expect cleft palate to occur with a clearly higher incidence than anophthalmia and an even higher incidence than microphthalmia. But prothioconazole caused no cleft palate and no anophthalmia (in both rat dev tox studies, the rat 2-gen study and the rabbit dev tox study) and it caused no microphthalmia in the supplementary rat dev tox study, and in the rabbit dev tox study.*

Based on these considerations it is concluded that the isolated finding of unilateral "small eye) in one high-dose pup of the two-generation study does not indicate a treatment-related case of microphthalmia. The NOAEL for microphthalmia in this study is therefore concluded to be the highest tested dose, 750 mg/kg bw/d.
Besides these conclusions, it is evident that the clinical observations applied in the two-generation study were able to detect abnormal eyes. This increases the value of this study to clarify (in addition to the supplemental developmental toxicity study in the same strain) "semi-mechanistically" that the microphthalmia observed in the first rat developmental toxicity study does not indicate a specific teratogenic potential of prothioconazole.

RAC's response

Your opinion is noted, thank you. RAC acknowledges that this is a case between no classification and classification in category 2, that will be discussed in the ODD. RAC agress with the DS that prothioconazole-desthio, a triazole as opposed to prothioconazole which is a triazolinethione, likelt does not have an impact on the incidence of micropthtalmia following prothioconazole exposure.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	17

Comment received

Skin sensitisation:
Two skin sensitisation assays were performed with prothioconazole: a Maximisation assay using a batch of high purity (99.8%) and a modified LLNA assay using a batch with a purity of 97.2% and containing sensitising impurities (information from the Renewal Assessment Report, UK, 2018). The LLNA assay was a modified test not validated at European/international level. Therefore, information on the validity of this test should be provided, taking into account requirements of Annex I of OECD TG 429 (2010)? If, in view of these validity data, this modified LLNA assay could not be considered as acceptable, classification of prothioconazole active substance as a skin sensitiser should be

considered, taking into account the presence of sensitising impurities in the current specifications and the absence of any impurity in the negative Maximisation test.

Dossier Submitter's Response

A guinea-pig maximisation study was submitted for the original approval of prothioconazole as a pesticide active substance to inform on the skin sensitisation potential of prothioconazole; this study was conducted on a pilot-plant batch with a high purity. For commercial, large-scale production, a new production pathway resulted in a modified specification with an increase in the amount of one impurity and introduction of another impurity, both of which have mild skin sensitising properties. To address this potential concern, the applicant submitted a new mouse LLNA performed on the currently-proposed specification of prothioconazole. This assay was deemed to be acceptable for the meeting of the relevant information requirement for the renewal of approval of prothioconazole as a pesticide active substance. The modifications applied have been extensively investigated and reported in the published literature and shown to have comparable sensitivity to the 'standard' LLNA (Vohr et al. (1994), Ikarashi et al. (1993)). Furthermore, the measurement of ear swelling after treatment is included, leading to a more simplified and reliable assay (Homey et al. (1998)). By comparing the specific immune reaction induced by the test item in the draining lymph nodes (LN; cell counts / LN weights) with the immediate unspecific acute skin reaction (ear swelling / ear weight) it is possible to discriminate the irritant potential from the sensitising potential of the compound tested. Methodological reliability and sensitivity is confirmed by supplementary studies in regular intervals in the performing laboratory. In the prothioconazole study, the mice did not show any increase in the stimulation indices for cell counts or for weights of the draining lymph nodes following epicutaneous application of up to and including 50 % of the test item for 3 consecutive days onto both ears of the animals.

In conclusion, both tests were clearly negative, indicating that prothioconazole did not exhibit a skin sensitisation potential under these test conditions.

Furthermore, one of the impurities that is known to be a skin sensitiser is present in the proposed technical specification at a level that is below the generic concentration limit for classification in accordance with CLP (Renewal Assessment Report Volume 4, 2018).

Overall, we conclude that prothioconazole should not be classified for skin sensitisation.

Vohr et al.: Detection of photoreactivity demonstrated in a modified local lymph node assay in mice. Photoderm. Photoimm. & Photomed., 10, 57 (1994).
Ikarashi et al.: A sensitive mouse lymph node assay with two application phases for detection of contact allergens. Arch. Toxicol., 67, 629-636 (1993).
Homey et al.: An integrated Model for the Differentiation of Chemical-Induced Allergic and Irritant Skin Reactions (IMDS). Toxicol. and Appl. Pharmacol., 153, 83-94 (1998).
Vohr, H.-W., et al.: An intra-laboratory validation of IMDS: Discrimination Between (Photo)Allergic and (Photo)Irritant Skin Reactions in Mice. Arch. Toxicol., 73, 501-509 (2000).

RAC's response

Your opinion is noted, thank you. RAC agrees that the modified mouse LLNA can be considered reliable. Based on two negative skin sensitisation studies, RAC agrees that prothioconazole should not be classified for skin sensitisation.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2018	Belgium		MemberState	18

Comment received

In the key aquatic Chronic study performed with Skeletonema costatum (Kern and DeHaan, 2004) not all validity criteria were met at 96h. Therefore the results are not reliable.
What was the % of mean section-by-section growth rate coefficients of variation at 96h in the control and solvent control?
The 72hErC50/ErC10 was calculated on the basis of the 96hr ratio between the nominal and the mean measured concentrations. As you mention in the CLH report this is not a completely accurate method for calculating the endpoints. However it is not clear for us why the mean measured value of the 72hErC50/ErC10 wasn't used for classification, because all validity criteria were met at 72h.

Can you confirm that exponential growth of algae was maintained throughout the test period under the prevailing conditions?

Can you please clarify which solvent was used and which concentration was applied. Is there an explanation for the very high growth observed in the solvent control. Why were control data pooled (high variation, …)?

Some editorial or/and minor comments :
Typo on p.75, 1st paragraph after table 43 : ErC50=0.03587 should read ErC50=0.03278

Table 41 mentions for Prothioconazole-desthioa a 96hLC50 of 10.4 mg p.m./L (mm) for Leuciscus idus melanotus while in the description on p.71 a LC50 = 6.63 mg p.m./L is reported. Idem for the study with Procambarus clarkia where in the table a LC50 >26 mg p.m./L (mm) is mentioned, while in the description the LC50 is said to be 0.069 mg p.m../L.

Dossier Submitter's Response

1) The following validity criteria in the Kern and DeHaan (2004) study were not met:

- Negative control mean section-by-section growth rate coefficient of variation was not <35% at 96 hours (49%; excluding replicate A, discussed further below)
- Solvent control mean section-by-section growth rate coefficient of variation was not <35% at 96 hours (55%)

One of the replicates in the control group experienced abnormally low growth and was thus excluded from the calculations; therefore only 2 control replicates were valid. All three solvent control replicates demonstrated very strong growth and therefore the control data were pooled, therefore, the limited number of valid negative control group replicates is not considered to impact on the validity of the endpoints.
As the 72 hour validity criteria were all met, it was concluded that the endpoints should be based on the effects at 72 hours.

2) As mentioned in the CLH report, the validity criteria for this study were met at 72h. 72h endpoints are considered valid endpoints to use from aquatic plant and algae studies. Ideally, the mean measured 72h E_rC_{50}/E_rC_{10} would have been taken from this study for

classification purposes. However, as stated in the CLH report, mean measured endpoints were only available for the 96h E_rC_{50}. In terms of nominal concentrations the 96h E_rC_{50}, and 72h E_rC_{50} were available. Therefore, in order to estimate the mean measured 72h E_rC_{50}, the RMS calculated the ratio between the nominal 96h E_rC_{50} and the mean measured 96h E_rC_{50}, and then multiplied the nominal 72h E_rC_{50} by this ratio. This is demonstrated in the following table taken from the CLH report:

Table 42: Estimation of 72 hour mean measured concentration E_rC_{50}

96 hours			72 hours		
Nominal E_rC_{50} (µg a.s./L)	Mean measured E_rC_{50} (µg a.s./L)	Ratio between nominal and mean measured endpoints	Nominal E_rC_{50} (µg a.s./L)	Calculation	Estimated mean measured E_rC_{50} (µg a.s./L)
49.9	35.87	0.719	45.6	45.6 x 0.719	32.78

This estimated mean measured E_rC_{50} *was* used as the basis of the acute aquatic hazard classification in the draft CLH report, as it was the lowest acute aquatic endpoint, resulting in a classification of category acute 1. To quote the CLH report, page 84: "The lowest value is 0.03278 mg/L for the marine diatom Skeletonema costatum. On this basis, prothioconazole meets criteria from the CLP directive (Annex I, section 4.1, table 4.1.0) for classification in Category Acute 1."

In addition, $EC_{10}/_{20}$ values based on mean measured concentrations were only available at 96h. Thus, to estimate the 72h $EC_{10}/_{20}$ values, the ratio between the 72h and 96h EC_{50} value was calculated and then multiplied by the 96h EC_{10} and EC_{20} values, respectively. This is demonstrated in the following table taken from the CLH report:

Table 43: Estimation of 72 hour mean measured concentration E_rC_{10} and E_rC_{20} values

Endpoint	96 hours		72 hours	
	Mean measured endpoint (µg a.s./L)	Ratio between 72 hour and 96 hour EC_{50} endpoint	Calculation	Estimated mean measured endpoint (µg a.s./L)
EC_{10}	15.62	0.9139	15.62 x 0.9139	14.27
EC_{20}	20.84		20.84 x 0.9139	19.04

The estimated EC_{10} *was* used as the basis of the chronic aquatic hazard classification in the draft CLH report, as it was the lowest chronic aquatic endpoint, resulting in a classification of category chronic 1, M-factor = 1. To quote the CLH report, page 85: "The lowest chronic endpoint is for algae, i.e. the 72 hour E_rC_{10} growth rate endpoint = 0.01427 mg a.s./L. As this endpoint is <0.1 mg/L and as prothioconazole is considered not rapidly degradable, the corresponding chronic classification is Chronic Category 1. The relevant Chronic M-factor is 1."

It is noted that in the summary for this study in the draft RAR for the active substance, the RMS has stated that "the Applicant will be requested to calculate these (*i.e. 72h*) endpoints on mean measured concentrations." Therefore these endpoints may change

and the CLH report may need amending following the formal commenting stage of the draft RAR for the active substance.

3) The study summary in the RAR reports that the negative control mean biomass increased by more than a factor of 16 within 72 hours, and the solvent control biomass increased by more than a factor of 16 within 72 hours, therefore it is concluded that exponential growth of algae was maintained throughout the initial 72 hours of the test.

4) The positive control was treated with acetone at 0.5 mL/L. This was the same as the solvent concentration used in the test substance treatments. The study report states that no statistically significant differences were noted between the control and solvent control groups for any of the 72 hour and 96 hour parameters, therefore the controls were pooled for statistical comparisons. However, it is noted that replicate A of the negative control was excluded from all calculations due to reduced growth. As the results from this replicate demonstrated very little growth had occurred, the RMS evaluator concluded that it was acceptable to treat this replicate as an anomaly and discount it from calculations in the draft RAR of the active substance. The reduced number of negative control replicates was not considered to impact on the validity of the endpoints as the negative and solvent control data was pooled for evaluation purposes. The (unacceptably) high variation apparently occurred after the 72 hour period, as the CoV was in the acceptable range for both controls at 72h but not at 96h. As mentioned, the 72h endpoints were therefore considered to be the acceptable endpoints from this study.

5) Agreed. This can be amended to E_rC_{50}=0.03278.

6) Agreed. The LC_{50} for *Leuciscus idus melanotus* on page 71 can be amended to 10.4 mg p.m./L (mm). The LC_{50} quoted on page 73 for *Procambarus clarkii* can be amended to > 26 mg p.m./L.

RAC's response

Thank you for your comment.

RAC appreciates the clarification provided by the DS regarding the key aquatic chronic study performed with Skeletonema costatum (Kern and DeHaan, 2004).

RAC noted that in the CLH report and in the DS's response, there is a reference that "*the RMS has stated that the Applicant will be requested to calculate these (i.e. 72h) endpoints on mean measured concentrations*", therefore RAC asked EFSA if these additional recalculations for the algae *S. costatum* have been generated by the Applicant and were made available to them. In the response, EFSA explained that no request to recalculate the endpoints for this species was finally sent to the Applicant, therefore no additional calculations were performed by the Applicant.

The editorial mistakes are noted.

Date	Country	Organisation	Type of Organisation	Comment number
06.06.2018	Netherlands		MemberState	19

Comment received

NL agrees with the proposed classification and M-factors for the aquatic toxicity of prothioconazole. The fact that the chronic toxicity of the degradation product, prothioconazole-desthio, is almost a factor 10 higher is sufficiently taken into account in

the proposal. Because the substance is considered as not rapidly degradable which results in M-factors being a factor 10 higher..
Dossier Submitter's Response
Noted, thank you for your support.
RAC's response
Thank you for your comment. RAC notes the support for the proposed environmental classification.

Date	Country	Organisation	Type of Organisation	Comment number
19.06.2018	France		MemberState	20
Comment received				
FR agrees with the classification and M factor values proposed for Environmental hazards.				
Dossier Submitter's Response				
Noted, thank you your support.				
RAC's response				
Thank you for your comment. RAC notes the support for the proposed environmental classification.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2018	Germany		MemberState	21
Comment received				

Section 8.7 Self-reactive substances:
The conclusion on classification and labelling for self-reactive substances based on test results according to Method EC A.16 is not valid. We assume that self-heating has been confused with self-reactive (cf. Table 7 and Table 17 of the CLH report).

We propose to replace the evaluation on self-reactive substances in section 8.7 as follows:
Prothioconazole undergoes thermal decomposition which has been experimentally verified by OECD 113 using a differential scanning calorimetry (DSC) (cf. section 8.1 Explosives). According to the provided data in section 8.1 the classification as explosives can be excluded. Therefore, an assessment for self-reactive need to be performed.

Data waiving may be acceptable in accordance with the given definition of self-reactive substance in section 2.8.2.1 of Annex I to Regulation (EC) No 1272/2008:

(a) they are explosives, according to the criteria given in 2.1;
(b) they are oxidising liquids or solids, according to the criteria given in 2.13 or 2.14, except that mixtures of oxidising substances, which contain 5% or more of combustible organic substances shall be classified as self-reactive substances according to the procedure defined in 2.8.2.2;
(c) they are organic peroxides, according to the criteria given in 2.15;
(d) their heat of decomposition is less than 300 J/g; or
(e) their self-accelerating decomposition temperature (SADT) is greater than 75°C for a 50 kg package (See UN RTDG, Manual of Test and Criteria, sub-sections 28.1, 28.2, 28.3 and Table 28.3.)
The exothermic decomposition energy of Prothioconazole was measured and determined

to be > 1013 J/g. Prothioconazole started to decompose at 215 °C. An experimentally study to determine the self-accelerating decomposition temperature (SADT) in a 50 kg package was not performed. However, due to the melting point of 140.3 °C and the onset temperature above 200 °C it can be concluded that the SADT is greater than 75°C for a 50 kg package and that therefore, Prothioconazole does not meet the criteria to be classified as a self-reactive substance.
Dossier Submitter's Response
Thank you for your comments. We agree that the available data indicate that the SADT would be >75°C and therefore the substance does not have to be considered for classification in this hazard class.
RAC's response
Noted

PUBLIC ATTACHMENTS
1. BMD_public.zip [Please refer to comment No. 11]
2. PTZ is a triazolinethione and does not cause the reprotox observed for several classical triazoles_sanitized.pdf [Please refer to comment No. 6]

CONFIDENTIAL ATTACHMENTS
1. BMD_confidential.zip [Please refer to comment No. 11]