Committee for Risk Assessment

RAC

Opinion
proposing harmonised classification and labelling
at Community level of
fuberidazole

ECHA/RAC/ CLH-O-0000001404-79-01/F

Adopted

26 October 2010

Annankatu 18 | P.O. Box 400 | 00121 Helsinki | Finland
www.echa.eu | Tel.: + 358 9 68.61.80

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT COMMUNITY LEVEL

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

Substance Name: ***Fuberidazole***

EC Number: ***223-404-0***

CAS Number: ***3878-19-1***

The proposal was submitted by ***United Kingdom*** and received by ECHA on ***17 November 2009***

The proposed harmonised classification by United Kingdom

	Directive 67/548/EEC (criteria)	CLP Regulation (EC) No 1272/2008
Current entry in Annex VI CLP Regulation	Xn; R22 N; R50-53 (Table 3.2)	Acute Tox. 4 H302 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 (Table 3.1)
Current proposal for consideration by RAC	Xi; R43 Xn; R48/22	Skin sens. 1; H317 STOT RE 2 (heart); H373
Resulting harmonised classification, proposed future entry in Annex VI CLP Regulation.	Xn; R22 Xi; R43 Xn; R48/22 N; R50-53	Acute Tox. 4; H302 Skin sens. 1; H317 STOT RE 2 (heart); H373 Aquatic Acute 1; H400 Aquatic Chronic 1; H410

PROCESS FOR ADOPTION OF THE OPINION

United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was

made publicly available in accordance with the requirements of the CLP Regulation at http://echa.europa.eu/consultations/harmonised_cl/harmon_cl_prev_cons_en.asp on 18 January 2010. Parties concerned and MSCAs were invited to submit comments and contributions by 3 March 2010.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Norbert Rupprich***

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

The RAC opinion on the proposed harmonised classification and labelling has been reached on ***26 October 2010,*** in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2.

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

OPINION OF RAC

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

Classification & Labelling in accordance with the CLP Regulation:

Classification:	Acute Tox. 4 - H302 Skin Sens. 1 - H317 STOT RE 2 - H373 May cause damage to organs (heart) through prolonged or repeated exposure. Carc. 2 - H351 Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410
Specific concentration limits:	-
M-factors:	The M factor is 1 based on 0.1 <L(E)C_{50} ≤1 mg/l
Notes:	None
Labelling:	GHS07,GHS08,GHS09 Wng. H302, H317, H373, H351, H410

Classification & labelling in accordance with Directive 67/548/EEC	
Classification:	Xn; R22 Xi; R43 Xn; R48/22 Xn; R40 (Carc. Cat. 3) N; R50/53
Specific concentration limits:	
	N; R50/53: C>= 25% N; R51/53: 2.5 % <= C < 25 % R52/53: 0.25 % <= C < 2.5 %
Notes:	None
Labelling:	Xn; N R: 22-43-48/22-50/53 S: (2)-22-36/37-60-61

SCIENTIFIC GROUNDS FOR THE OPINION

Fuberidazole is a benzimidazole fungicide

Fuberidazole is a benzimidazole fungicide that is used as a seed treatment. In 2008 it was approved for Annex I listing as a 3A Review compound under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, fuberidazole should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points.

The original dossier submitter's proposal did not contain a classification proposal for carcinogenicity. RAC concluded that comparison of available carcinogenicity data with corresponding classification criteria warrants an additional classification for carcinogenicity.

General remarks

Comments received during the public consultation have been taken into account in this draft opinion.

The following part of the Opinion Document essentially is a targeted summary of the corresponding Background Document. This summary mainly corresponds to the endpoint-related "summary and discussion" chapters of the Background Document. Thus this summary

concentrates on the most important experimental results, the history of decision finding and the final RAC proposal. Compared to the Background Document, this Opinion Document does not contain any additional information.

Acute toxicity

Fuberidazole is listed on Annex VI of the CLP Regulation. It was inserted into Annex I of Directive 67/548/EEC in the 19th ATP in 1993 with the acute toxicity classifications of Xn; R22. The LD_{50} values (range from > 300 to 792 mg/kg) obtained from three acute oral toxicity studies were within the range (200-2000 mg/kg) for Xn; R22 (criteria in Directive 67/548/EEC). No information opposing this classification was received during the public consultation and RAC discussion. Thus, based on the data available it was confirmed by RAC that fuberidazole meets the criteria for the current classification for acute toxicity (Xn; R22 respectively Acute Tox. 4; H302).

Irritation

The available information does not indicate that fuberidazole is irritant to the skin, the eye or the respiratory tract. No information opposing this evaluation was received during the public consultation and RAC discussion. Thus, based on the data available it was confirmed by RAC not to propose a classification for irritation.

Sensitisation

Fuberidazole was positive in a guinea pig maximisation test but negative in a guinea pig open epicutaneous test. With reference to the CLP guidance, the guinea pig maximisation test is used for classification purposes. Given the clearly positive findings in the maximisation test (i.e. clear responses in greater than the required 30% of animals), a classification of Xi; R43 under Directive 67/548/EEC and of skin sensitisation category 1 (H317) under the CLP Regulation is proposed. No information opposing this evaluation was received during the public consultation and RAC discussion. Thus, based on the data available RAC recommends the proposed classification for skin sensitisation.

There is no available information on the potential of the test substance to induce respiratory sensitisation.

Repeated Dose Toxicity

Fuberidazole has been orally tested for repeated dose toxicity in rats and dogs. Toxicological findings in the oral rat studies do not warrant a classification for repeated dose toxicity. However, based on the focal fibrosis of the heart observed in a one-year oral dog study at doses of 3.6 mg/kg/d and above, a classification of Xn; R48/22 (Directive 67/548/EEC) and STOT RE 2; H373 (CLP Regulation) has been proposed and justified by the dossier submitter. No classification has been proposed for the dermal or inhalation routes.

Public consultation and RAC discussions

In the public consultation and during RAC discussions there were comments proposing a more stringent classification (T; R48/25 or STOT RE 1 (heart); H372) for repeated dose toxicity, because there is already significant heart fibrosis in dogs at 3.6 mg/kg/d, a dose level below the default guidance values for a 90-day oral rat study of 5 mg/kg/d (67/548/EEC) and 10 mg/kg/d (1272/2008/EC).

RAC considered the following aspects when deciding between the two possible categories for repeated dose toxicity classification:

Myocardial fibrosis in the 1-year dog study

Histopathology revealed a dose-related increase in the incidence and severity of focal fibrosis of the papillary muscles of the heart (1-year dog study). The corresponding LOAEL is 3.6 mg/kg/d. Gross cardiac changes were prominent at the high-dose level of 36 mg/kg/d. There was one substance-related death at the high-dose level of 36 mg/kg/d. The remaining animals did not exhibit clinical signs of toxicity or illness, even at the highest dose. Thus there was a discussion at RAC whether the LOAEL of 3.6 mg/kg/d with myocardial lesions, but without recognised impaired heart function, is to be considered the effective dose for repeated dose toxicity classification:

7-day and 29-day dog study

RAC discussed on how to account for the results of the 7-day and 29-day dog studies:

In a 7-day dog study one animal of the 200 mg/kg/d group died on day 2. This animal had sub-endocardial haemorrhages and early autolytic changes but no evidence of myocardial lesions. In the survivors, ECG, blood pressure, pulse rate and histopathological investigations of the left ventricular papillary muscle gave no indications of a specific cardiotoxic effect.

In a 29-day oral dog study there was severe general and liver toxicity at the highest dose of 200 mg/kg/d. At 200 mg/kg/d blood pressure and pulse rates were reduced. ECG did not show any effects on the heart function. There was no evidence of myocardial damage (histopathology by light and electron-microscopy, electrocardiographic monitoring). This was supported by there being no change in the creatinine kinase levels in any of the groups. Compared to the 1-year dog study, the highest dose level in the 29-day dog study was about 5-times higher; while the duration of exposure was about 12-times lower.

Obviously in these short-term dog studies at 200 mg/kg/d there were no myocardial lesions. Whether reduced blood pressure and reduced pulse rates are related to cardiotoxicity has not been sufficiently analyzed. RAC concluded that the differences in cardiotoxicity in the short- and long-term study should not be considered contradictory or inconsistent, because a clear dependency of the manifestion of myocardial lesions on duration of exposure is a plausible explanation. Therefore the results of the 29-day dog study should not be used for limiting the importance of the results of the 1-year dog study for classification purposes.

Potential species differences

There is some evidence of species differences with regard to heart toxicity: while the dog shows heart fibrosis in the 1-year study at dose levels between 3.6 and 36 mg/kg/d, there was no finding of cardiac toxicity in the 4-month rat feeding study up to doses of about 400 mg/kg/d (in the DAR there is the information that for this rat study histopathology of the heart was performed). No heart lesions were reported in the 2-year rat and mouse carcinogenicity studies with the highest dose level of 155 mg/kg/d in rats and 551 mg/kg/d in mice (following RAC 12 the dossier submitter confirmed that histopathology of the heart was conducted in both the carcinogenicity studies). Thus RAC recognised that there is some experimental evidence for species differences (rat, mouse, dog). However, because it is not known for this substance which animal species is the most relevant to humans, classification is to be based on the more severe effects in dogs.

Guidance values

The CLP guidance values (dose level of 10 mg/kg/d for the borderline between STOT RE 1 and RE2) refer to significant/severe adverse effects in a standard 90-day oral rat study. In the CLP guidance it is outlined as well that this guidance value can be used as a basis to derive equivalent guidance values for toxicity studies of greater or lesser duration of exposure. However, there is no guidance as to the use of these rat-specific guidance values for studies with other experimental species (such as dogs). In 2006, NL presented a corresponding thought starter (ECBI/64/06) with considerations on how to translate guidance values for the rat to guidance values to dogs based on allometric scaling and different lifespans of species. However, these prelimanary discussions on the use of allometric scaling and different lifespans of species for RDT classification have not yet been finalized and the corresponding concepts have not yet been integrated into the CLP guidance. Thus for now RAC prefers to generally start with the guidance values for the 90-day oral rat study, to adapt these 90-day rat guidance values for different durations of exposure to rats and then to use the original or duration-adjusted rat guidance values without further changes for test results with other animal species.

Correspondingly, RAC supports the following basic rule for the borderline between STOT RE 1 and RE 2 for rats: based on “10 mg/kg/d for a 90-day study” the guidance value for a 28-day study is set to be higher (e.g. 30 mg/kg/d); the guidance value for a study with longer duration is considered to be lower (e.g. 2.5 mg/kg/d for a 1-year study). Thus, as a default, RAC proposes to compare the effective dose in the 1-year dog study with a CLP guidance value of 2.5 mg/kg/d. The corresponding DSD guidance level for the 1-year dog study (separating R48/22 from R48/25) is 1.25 mg/kg/d (5/4).

RAC conclusion

The data and considerations on the myocardial lesions in the 1-year dog study, the results in the 7-day and 29-day dog studies, the potential species differences and the concept on how to generally define guidance values for animal species other than rats pinpoint the relevant basis for decision finding:

RAC recognises that for the LOAEL of 3.6 mg/kg/d with histopathological heart lesions there was no experimental evidence of clear functional disturbance (1-year dog study). Not really knowing the relative relationship between histopathological heart lesions and functional consequences in dogs and humans RAC considers the histopathological lesions at 3.6 mg/kg/d

as relevant and convincing on its own and considers the LOAEL of 3.6 mg/kg/d as effective dose for repeated dose toxicity classification.

As a default, the modified CLP guidance value for a 1-year oral rat study of 2.5 mg/kg/d (10/4) is directly used for the results of the 1-year dog study as well.

Comparing the effective dose of 3.6 mg/kg/d with the CLP guidance value of 2.5 mg/kg/d, and being aware of the discussed data on the relationship of histopathological heart damage and functional disturbance, on the dependency of heart lesions in dogs on duration of exposure and on the possible species differences, RAC is in favour of STOT RE 2 rather than STOT RE 1. Correspondingly, RAC is in favour of R48/22 rather than R48/25.

Finally, following a detailled discussion of the fuberidazole data on repeated dose toxicity and the default guidance levels to be preferred for animal species other than rats, RAC supports the original proposal of the dossier submitter (CLP STOT RE2 (heart); H373 and DSD Xn; R48/22).

Mutagenicity

Fuberidazole was a clastogen in vitro in the presence of metabolic activation. However, it demonstrated no clastogenic activity in vivo, and so the in vitro finding is not considered to be of relevance to humans. Despite the spindle-inhibiting properties of benzimidazoles, fuberidazole did not show any aneugenic potential either in vitro or in vivo.

No classification has been proposed by the dossier submitter under Directive 67/548/EEC or the CLP Regulation. No information opposing this proposal was received during the public consultation and RAC discussion. Thus, based on the data available it was confirmed by RAC not to propose a classification for germ cell mutagenicity.

Carcinogenicity

Rats and mice were tested in combined oral chronic/carcinogenicity studies. In these studies there were statistically significant increases of incidences of the following tumour types:

- Hepatocellular adenoma in male mice
- Endometrial adenocarcinoma in the uterus of female rats
- Follicular cell adenoma in the thyroid of female rats

The dossier submitter concluded that the available evidence on the carcinogenic potential of fuberidazole in rats and mice is not sufficiently relevant for humans and does not warrant a classification of fuberidazole for carcinogenicity.

Public consultation and RAC discussions

Some comments received during public consultation and RAC discussion supported this original line of justification, other comments questioned the proposed classification. In order to facilitate decision finding the discussion in RAC was structured along the relevant tumour

types with significantly increased tumour incidences: namely hepatocellular adenomas in male NMRI mice, endometrial adenocarcinomas and follicular cell adenomas in female Wistar rats.

Hepatocellular adenomas in male (and female) NMRI mice

The incidence of hepatocellular adenomas in male NMRI mice is increased compared to the concurrent controls (2, 2, 6, 24%* at 0, 100, 600, 1800 ppm; feeding study). The statistically significant increase is restricted to the high dose level. The dose response is monotonic, thus considered to be a clear dose response.

The increased incidence of benign tumours is outside historical control ranges (0 to 18%; 2 to 16%) from different laboratories. The tumour incidences in the concurrent control group (2%) and the low dose group (2%) lie at the lower end of the historical control range (0 to 18%). The historical control range reported indicates some kind of intermediate susceptibility of male NMRI mice for liver tumour formation (compared e.g. to male B6C3F1 mice).

The hepatocellular adenomas induced by exposure to fuberidazole are observed only parallel to increased relative weight and focal liver necrosis with dose-related incidences of 14, 6, 38 and 48%. Fuberidazole is not considered to be mutagenic in vivo. Liver tumour formation therefore might be a secondary consequence of liver toxicity. However, it has to be recognised that (1) there are no data on the dose-related severity of the focal liver necrosis, (2) there is no information whether the animals with liver necrosis developed the liver tumours and (3) liver toxicity in subchronic studies did not fulfill the criteria for repeated dose toxicity classification.

For comparison: The incidence of hepatocellular adenomas in female NMRI mice is slightly increased compared to the concurrent controls (0, 0, 2, 4% 0, 100, 600, 1800 ppm). There is no statistical signifance.The increased incidence is just inside the historical control range (0 to 2%; 0 to 4%). The historical control range reported only indicates a low susceptability of female NMRI mice for liver tumour formation. Yet, the low incidence of tumours induced by exposure to fuberidazole is observed only in the presence of clear hepatotoxicity (focal liver necrosis with dose-related incidences of 12, 20, 30 and 28%).

Endometrial adenocarcinomas in Wistar rats

The incidence of endometrial adenocarcinomas in female Wistar rats was increased compared to concurrent controls (0, 8, 4, 10%* at 0, 80, 400, 2000 ppm). The dose response is not monotonic, thus not being a strict dose response. There were no cervical adenocarcinomas.

The increased incidence is near the upper level of relevant (same lab) historical control ranges (0 to 8% for combined uterine and cervical adenocarcinomas; 2 to 10% for uterine adenocarcinomas only). The tumour incidence in the concurrent control group (0%) lies at the lower end of the historical control range (0 to 10%).

With reference to the potential mode of action the findings in the 1-year dog study (e.g. increased uterus weight) might give some evidence of possible endocrine effects of fuberidazole. It is known that many azoles do affect the endocrine system. Following RAC 12

the dossier submitter confirmed that uterus weight was not determined in the rat carcinogenicity study. In the mouse carcinogenicity study, uteri were examined by histopathology, with no incidences of endometrial adenocarcinomas; but uterus weights were not measured as well. Based on these data, for the endometrial adenocarcinomas in Wistar rats a mode of action cannot be described.

Follicular cell adenomas of female Wistar rats

The incidence of follicular cell adenomas in female Wistar rats was increased compared to concurrent controls (0, 2, 0, 8% at 0, 80, 400, 2000 ppm). There was a statistically significant positive trend ($P<0.05$), but no statistical significance in pair-wise comparisons. The dose response was not monotonic, thus not to be considered a strict dose response.

The high-dose incidence (8%) is outside of relevant (same lab) historical control ranges (0 to 2%). The other set of historical control data is not considered sufficiently representative (0 to 6.5%). These latter data are from different laboratories; furthermore, following RAC 12 the dossier submitter clarified that this latter set of historical control data covers a time period of 20 years before the fuberidazole study, with the 6.5% study in 1975 (the fuberidazole study was published in 1993).

There are no further lesions in the thyroid gland (weight, histopathology) that may indicate a significant increase of thyroid activity. At the high dose level liver hypertrophy is described for both male and female rats; however, it is not known whether there was an induction of the UDP glucuronyl transferase which is responsible for the metabolism of T4. It is well known that rats are more susceptible to tumour development resulting from thyroid gland stimulation than humans. However, this mode of action has not been sufficiently demonstrated for fuberidazole. Fuberidazole is not mutagenic in vivo. There are no further data available allowing for a positive description of the mode of action.

CLP guidance on the relevance of mode of action

The CLP guidance (chapter 3.6.2.3.2(k)) comments on the importance of information on the mode of action of tumour development. Only if a mode of action of tumour development is conclusively determined not to be operative in humans then the carcinogenic evidence for that tumour may be discounted.

In 1999 specialised experts specifically commented on the classification of substances causing thyroid tumours in rodents. For non-genotoxic compounds and known disturbance of the thyroid-pituitary axis classification was recommended based on the experimental carcinogenic potency. For substances (with thyroid tumours in rodents) with unknown mechanism Carc. Cat. 3 (DSD) was recommended (ECBI/49/99 Add.1 Rev.2).

Comparison of fuberidazole data with the classification criteria for carcinogenicity

Comparison of fuberidazole carcinogenicity data with the corresponding classification criteria is not trivial because the data are complex and some kind of borderline and the criteria leave a margin for different interpretations. To start with RAC recognises that there are statistically

significant increases in tumour incidences for three types of tumours (malignant and benign) in two different species. This is generally taken as positive evidence of carcinogenic activity.

However, this overall description needs to be differentiated: the dose response for the endometrial adenocarcinomas is not monotonic; the high-dose level incidence of 10% is at the upper limit of historical control data. The dose response for the follicular cell adenomas is non-monotonic as well; however, the high-dose level incidence of 8% is beyond the relevant upper historical control range of 2%. The dose response for the hepatocellular adenomas is monotonic; the high-dose level incidence of 24% is outside the upper range of available (different labs) historical control data. Spontaneous liver tumour incidences in male NMRI mice are lower than those in male B6C3F1 mice. Overall, it is the opinion of RAC, that concurrent controls should be the main reference for comparison with tumour incidences in treated animals, with historical control data used as an additional refinement.

Considerations on mode of action are specifically relevant for the issue of carcinogenicity classification. For the endometrial adenocarcinomas the possibility of endocrine effects was mentioned; however, available data do not allow for a corresponding conclusion. For the follicular cell adenomas, it cannot be excluded that fuberidazole had an effect on the rat thyroid-pituitary axis; again, available data do not document this mode of action. Hepatocellular adenomas might be secondary to liver toxicity; but again, the experimental evidence is not sufficient to conclusively clarify the liver-related mode of action. Overall, it has to be recognised, that the fuberidazole-related information on the carcinogenic mode of action is scarce and does not allow for firm conclusions.

It is considered evident by RAC that such a tumour profile does not allow for a CLP 1B category; mainly because of the rather weak dose-response relationship for all three types of tumours in combination with the missing in vivo genotoxicity.

The remaining question however is, whether the data available are sufficiently positive for a CLP Cat 2 category or, respectively, are sufficiently negative for not classifying fuberidazole for carcinogenicity. RAC recognized that (1) there is a statistically significant increase of three types of tumours in two species, (2) although in general dose response relationships are weak, there remains a recognisable experimental carcinogenic potential of fuberidazole, (3) the information on the possible modes of action and its relevance to humans is scarce and cannot be used to dismiss the available carcinogenicity data. Only in case of verified modes of action with an overly susceptability in a tested species versus humans there is the recommendation of no classification at all.

Reference to the original proposal of the dossier submitter

Endometrial carcinomas in Wistar rats: the dossier submitter did not consider the data on the endometrial adenocarcinomas in Wistar rats as sufficient evidence of carcinogenicity. This mainly because of the shape of the dose response (“no clear dose-response relationship”) and the comparison with historical control values (“incidence … within the observed range of the historical control data ….”. RAC put more emphasis on the comparison of the treatment-related tumour incidences to the concurrent controls, additionally recognising that the high-dose incidence of 10% was not well within the historical control range, but at the upper level of the reported historical ranges (0 to 8% resp. 2 to 10%).

Hepatocellular adenomas in male NMRI mice: the dossier submitter stated that for this tumour type "a mechanism of tumour induction has been identified that cannot be extrapolated to humans", that this tumour type "is known to occur spontaneously with a high incidence" and "that benign tumours are common in the liver of rats and mice". RAC concluded, that, compared to the B6C3F1 mice, the spontaneous liver tumour incidence in male NMRI mice is clearly lower (roughly up to 20% for male NMRI mice compared to up to 60 % for B6C3F1 mice). Additionally, RAC does not possess reliable knowledge on the mechanism of fuberidazole-related tumour development in male NMRI mice and on the relative susceptability of humans; and thus is not able to reveal that these data cannot be extrapolated to humans.

Follicular cell adenomas in female Wistar rats: the dossier submitter stated that "the rat thyroid in particular appears to be far more susceptable to carcinogenicity induced by xenobiotics than does the human thyroid". RAC recognised that such clear-cut species differences apply to substances which specifically disturb the rat thyroid-pituitary axis. However, such amechanism for the fuberidazole-induced follicular cell adenomas has not been experimentally verified; because of this missing information RAC specifically referred to ECB document ECBI/49/99 Add.1 Rev.2, which recommended Carc. Cat 3 (DSD) for substances with thyroid tumours in rodents with unknown mechanism.

RAC conclusion

Based on the considerations above, recognising a weak experimental carcinogenic potential of fuberidazole without convincing data that this carcinogenic potential is not relevant to humans, RAC does not follow the recommendation of the dossier submitter not to classify fuberidazole for carcinogenicity. RAC concluded that the available data, compared with the classification criteria, justify to classify fuberidazole into the category "limited evidence of carcinogenicity" (CLP Carc. Cat. 2 with H351 and DSD Carc. Cat. 3 with R40):

Fertility Impairment (Reproductive Toxicity)

Fuberidazole did not demonstrate any adverse effects on fertility in a two-generation reproductive study in rats at doses of up to 132 mg/kg/d. Therefore, no classification for fertility effects has been proposed by the dossier submitter.

No information opposing this evaluation was received during the public consultation and RAC discussion. Thus, based on the data available it was confirmed by RAC not to propose a classification for fertility impairment.

Developmental Toxicity (Reproductive Toxicity)

Based on the developmental toxicity data available, comparing these data with the relevant classification criteria, the dossier submitter concluded that there is not sufficient and convincing evidence for a developmental toxicity classification for fuberidazole.

RAC discussion and conclusion on microphthalmia

During public consultation the issue was raised that a typical malformation for benzimidazole derivates, namely microphthalmia, occurred twice in the oral rat developmental toxicity study, one case in the low-dose group and another one in the mid-dose group. It was stated that a dose dependency could not be expected with rare malformations, with the remaining question, whether these two cases of microphthalmia could really be regarded as incidental and unaffected by treatment.

The dossier submitter added the information that the strain of Wistar rats used in that study is known to be susceptible to the induction of this malformation, with reported foetal incidences of 2% and litter incidences of 20% (following RAC 12 the dossier submitter confirmed that these numbers were upper values). The low- and mid-dose incidences of macrophthalmia in the fuberidazole study are calculated to be about 0.4% (1 case in about 250 foetuses). Considering this additional information on relevant historical control incidences RAC concludes that these isolated cases of microphthalmia could be considered as incidental and do not provide sufficient evidence for a developmental toxicity classification.

RAC discussion on reduced pup viability in the 2-generation rat study

The second issue that was raised in the public consultation and in RAC discussions is the finding of reduced pup viability in the two-generation oral rat study. The changes in the viability and lactation indices were not associated with overt maternal toxicity and therefore, referring to the comments, might justify a classification as Repr. Cat. 3; R63 (CLP Repr. Cat. 2; H361d).

In RAC discussions various comments referred to the reported changes of the viability index (PND 0-5) and the lactation index (PND 5-28) and its influence on classification. On the other hand the importance of these separated discussions of both indices was questioned because this sort of analysis might exaggerate the importance of whether the pups die just before or just after day 5. Because of these methodological discussions RAC considered it helpful to additionally integrate the viability and lactation index to an overall viability index covering the viability of pups from PND 0 to 28. This is done by simple multiplication of both indices (being aware of the experimental schedule that 5 days after birth the litters were reduced if necessary to eight animals). The body weight gain data in the following table have been checked, modified and confirmed by the dossier submitter following RAC 12.

Table xx: Overall pup viability index from the 2-generation rat study

F1a Dose (ppm)	BWG 4w	Viability index PND 0-5	Lactation index 1. PND 5-28	Overall viability PND 0-28
0		97.4	87.9	85.6
50	unaffected	99.1	88.2	87.4
250	unaffected	98.8	92.9	91.8
1250	unaffected	94.4	87.0	82.1

F1b Dose (ppm)	BWG 4w	Viability index PND 0-5	Lactation index 2. PND 5-28	Overall viability PND 0-28
0		97.2	92.6	90.0
50	92%	98.2	91.2	90.0
250	99%	95.0	80.7**	76.7
1250	87%	94.6	75.5**	71.4

F2a Dose (ppm)	BWG 4w	Viability index PND 0-5	Lactation index 3. PND 5-28	Overall viability PND 0-28
0		95.3	66.0	62.9
50	96%	82.8**	64.7	53.6
250	101%	94.7	86.2**	81.6
1250	88%	80.6**	56.3	45.3

F2b Dose (ppm)	BWG 4w	Viability index PND 0-5	Lactation index 4. PND 5-28	Overall viability PND 0-28
0		90.7	31.7	28.8
50	105%	86.5	48.5**	42.0
250	108%	82.1**	30.5	25.0
1250	91%	60.9**	35.8	21.8

Short summary of basic findings for pup viability

Fuberidazole was tested in a 2-generation rat feeding study. In the absence of relevant maternal toxicity there was a decrease of the lactation index in the F1b-generation and a decrease of the viability index in the F2b-generation (to a lower degree in the F2a-generation as well). These specific changes in pup viability were only observed in the generations specified, not in the remaining F1 or F2 generations.

When using the overall viability index (PND 0-28) (see table above) as a measure of pup viability it is again evident that there is no treatment-related effect on pup viability in the F1a generation. The overall viability index covers the range between about 80 and 90%. In the F1b generation there is a dose-dependent decrease of overall viability (90%, 90%, 77%, 71%). Overall viability of the F2a generation already starts with a low viability of pups even in the controls (63%) without any following dose-response relationship. The validity of results of the F2b generation is highly questioned because of an extremely reduced viability even in the controls (index of 29%).

Following RAC 12 the dossier submitter confirmed that industry historical control data for overall pup viability for the F1A pups was 70 to 100%, for the F1B pups 51 to 98% and for the F2A pups 61 to 87% (same laboratory, Fuberidazole study from 1985/86; control data from 1978 to 1989). The large variation of these historical control data for pup viability is recognised; because the reasons for this large variation are not really known, the comparison of the actual Fuberidazole data with these historical control data is only of very limited relevance.

Differing durations of exposure for different groups of dams

It has been discussed in RAC whether the difference of results for pup viability in the different generations can be considered consistent. The inconsistency discussion at least partly relates to the experimental schedule and the different durations of exposure to the various groups of dams. For the F1a generation there is the prebreed exposure period for the dams, and the prenatal and translactational exposure, with possible self-feeding of pups at the end of the lactation period. The difference to the F1b generation is, that the second mating results in a longer exposure period of the dams before the second gestation period. The main exposure-related difference between the F1 and F2 generations is, that dams of the F2 generation were already exposed to fuberidazole during its prenatal development. The impact of these different exposure schedules essentially might depend on the toxicokinetics of fuberidazole.

Based on the toxicokinetic section, it is known that fuberidazole elimination from the body is rapid; and that there is no indication that bioaccumulation of fuberidazole or its metabolites occurred. Pup viability in the different generations should be similar in case of similar blood levels of fuberidazole in the dams just before the corresponding gestation periods; only in case of clearly different "starting conditions" at the beginning of gestation for the different generations differences in adverse effects to the pups are considered plausible. Possibly there is not sufficient information to clarify this issue; nevertheless the differences in exposure schedules for the F1a, F1b, F2a and F2b generations did not result in any differences of pup viability and pup weight at birth. At least there is no clear positive indication or information why there should be substantial differences in pup viability in the 4 generations.

Potential infection during study

During RAC 11 industry raised the point of infections possibly being a cause for the substantial decrease of viability of pups especially in the F2 generations. Industry was asked to try to verify and document this suspicion. In June 2010 industry submitted supplemental information on the 2-generation rat study mainly relating to detailed historical control data and to the issue of possible infections of animals (Bayer CropScience 2010). Possible clinical signs of infection (bloody eye rims, dyspnoea), which are no signs of compound related toxicity due to the lack of any dose relationship, were observed in F1b parents at different time points of the study (in young F1b animals, before and after the first mating, during and shortly after the second mating). Only a small number of animals were affected, the symptoms were in some cases only temporary, and pathological examination did not detect any indications of an infectious disease in the animals. Nevertheless it is considered possible by industry that the lowered viability and lactation indices of the F2a and F2b generations both in the control and dose groups could be a consequence of the mainly subclinical infections of the parental animals.

RAC conclusion

In the 2-generation rat study treatment-related decreases of pup viability were reported. The interpretation of corresponding data is complicated by the fact (1) that the decreases of pup viability do not occur similarly in all 4 generations and (2) that there is an extremely high decrease of viability of pups in the F2 generation in control animals as well. Thus, initial differences in RAC opinion related to this issue of possible inconsistencies of findings and to the limitations of the study because of the unusual control data in the F2 generation.

With reference to the overall viability (PND 0-28) in the F1b generation there is a decreased viability of pups in the mid and high dose level, but there is no corresponding treatment-related effect in the F1a generation.
Because of the toxicokinetic properties of fuberidazole (no bioaccumulating potential) it is unlikely that the difference in exposure duration of dams can explain the observed differences in the adverse effects in the F1a and F1b generation.
It is a major limitation of the study that there is a very low control pup survival in both F2 generations. The reason for this is not known; it might be possible that subclinical infections of F1b parents are the cause for the peculiar changes especially of the lactation index in the F2a and F2b generation. This low control pup survival is considered to compromise the validity of the results of the F2 generations.
Thus, following a thorough discussion, it is the opinion of RAC that the findings on reduced pup viability in the F1a and F1b generation are not sufficiently consistent and therefore the weight of evidence is not sufficiently convincing for a developmental toxicity classification of the substance. The pup viability data of the F2 generations do not allow for a classification either because the assessment of these data is significantly limited by the finding of an unusual pup mortality even in the F2 control animals.

It needs to be additionally stressed that the available developmental toxicity studies do not indicate that fuberidazole is embryotoxic or teratogenic.

Thus RAC supports the original proposal of the dossier submitter not to classify fuberidazole for the toxicological endpoint of developmental toxicity.

Environment

Fuberidazole is listed on Annex VI of the CLP Regulation with the environmental classification of N; R50-53 (Directive 67/548/EEC) and Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 (CLP Regulation). The lowest acute toxicity value was a 96-h LC_{50} of 0.91 mg a.s./l for Oncorhynchus mykiss. Fuberidazole is not considered to undergo rapid and ultimate degradation under environmental conditions and is considered not readily degradable. Fuberidazole is considered to have a low bioaccumulation potential.

Based on the environmental data available compared to the relevant classification criteria the current fuberidazole classification “hazardous to the aquatic environment” is warranted. No information opposing this evaluation was received during the public consultation and RAC discussion. Thus, based on the data available RAC recommends to maintain the current classification for the aquatic environment.

Additional information

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

ANNEXES:

Annex 1 Background Document (BD)[1]

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information)

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