Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Bifenazate
EC number: 442-820-5
CAS number: 149877-41-8

CLH-O-0000003146-79-02/A2

Adopted

5 December 2013

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON BIFENAZATE (ISO);ISOPROPYL 2-(4-METHOXYBIPHENYL-3-YL)HYDRAZINECARBOXYLATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: bifenazate (ISO);isopropyl 2-(4-methoxybiphenyl-3-yl)hydrazinecarboxylate
EC number: 442-820-5
CAS number: 149877-41-8
Dossier submitter: The Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
07.05.2013	Belgium		MemberState	1
Comment received				
We would like to thank the Netherlands for the CLH report on Bifenazate.				
Dossier Submitter's Response				
-				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2013	United States	CHEMTURA CORPORATION	Company-Manufacturer	2
Comment received				
CHEMTURA BELIEVES THAT THE PROPOSED CLASSIFICATION OF STOT RE 2 IS NOT JUSTIFIED. *ECHA note: the confidential document will be provided as a separate document*				
Dossier Submitter's Response				
See response to comment 13.				
RAC's response				
See response to comment 13.				

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	3
Comment received				
The German CA supports the proposed classification and labeling as N; R50/53 (DSD) and H400, H410 (CLP regulation) also the acute and chronic M-factors and concentration limits. We support the following classification according to CLP Regulation as Skin sensitisation 1B and STOT RE 2, too. In addition, it would be helpful for clarification if the summary tables in the CLH report				

present study results as real values instead of information like 'decreased' or 'increased'.
Dossier Submitter's Response
Thank you for the support. We agree that quantitative data would be more valuable than qualitative data. However, unfortunately, such data are not always included in the DAR. Since the original studies are not available to us, we could not include the quantitative data in the CLH proposal.
RAC's response
The RAC is anticipated to perform an independent analysis and assessment of the data. Therefore the RAC agrees that quantitative data would certainly be more valuable than qualitative data.

Date	Country	Organisation	Type of Organisation	Comment number
30.04.2013	France		MemberState	4
Comment received				
FR agrees with the classification proposal on human health and the ecotoxicology/environment.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted. See the RAC opinion as well.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	5
Comment received				
Bifenazate has no carcinogenic potential. No classification is proposed.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted. See the RAC opinion for discussion of liver adenomas in male mice.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	6
Comment received				
Bifenazate has no mutagenic potential. No classification is proposed				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted. See the RAC opinion as well.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment

				number
08.05.2013	Germany		MemberState	7
Comment received				
Bifenazate has no reproductive potential. No classification is proposed				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted. Especially for developmental toxicity (retroesophageal aortic arch) see the RAC opinion as well.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	8
Comment received				
No specific data available				
Dossier Submitter's Response				
-				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Senzitation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
07.05.2013	Belgium		MemberState	9
Comment received				
We agree with the proposal to classify Bifenazate for skin sensitization based on the results of guinea pig maximisation test with an erythema seen in 17 out of 20 test group animals (85%).				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted. The RAC needs to clarify whether Skin Sens. 1 or Skin Sens. 1B is the optimal choice. See the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	10
Comment received				
Page 27-28: Bifenazate was tested negative in the Buehler test, but was tested positive (85 %) in the Maximisation test in Guinea pigs: According to Directive 67/548 EEC DSD Bifenazate should be classified as 'may cause sensitisation by skin contact (R43). According to CLP Regulation 1272/2008 Bifenazate should be classified as 'may cause an allergic skin reaction' (Skin sensitisation, cat 1B; H317).				

Dossier Submitter's Response
Thank you for the support.
RAC's response
Noted. The RAC needs to clarify whether Skin Sens. 1 or Skin Sens. 1B is the optimal choice. See the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Spain		MemberState	11
Comment received				
p. 27 Summary and discussion of sensitisation The Spanish CA supports the proposed classification of Bifenazate as skin sensitizer; R43 (May cause sensitisation by skin contact) according to Directive 67/548/EC and as Skin Sens. 1B (H317: May cause an allergic skin reaction) according to Regulation (EC) 1272/2008. This classification is based on the results of the Guinea Pig Maximisation Test (Rakhra and Donald, 2001) with Bifenazate (purity 90.4%) in which a positive response in 85% of the tested animals was observed (75% of the animals showed discrete or patchy erythema and 10% of the animals showed moderate or confluent erythema) after an intradermal induction dose of 6%.				
Dossier Submitter's Response				
Thank you for the support. The recent RAC conclusions and the updated draft guidance regarding sub-categorization shows that category 1B should only be applied if category 1A can be excluded. For bifenazate, category 1B cannot be excluded based on the results of the GPMT. However, the negative results of the Buehler test shows that bifenazate is not category 1A. Therefore, category 1A can be excluded based on the available skin sensitisation studies and category 1B is justified.				
RAC's response				
Noted. The RAC needs to clarify whether Skin Sens. 1 or Skin Sens. 1B is the optimal choice. See the RAC opinion.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
07.05.2013	Belgium		MemberState	12
Comment received				
We support the classification STOT RE based on o Changes in clinical haematology : in several studies, significant decrease haemoglobin, haematocrit, RBC, leukocytes, observed at low dose : - In 28 day diet in rats : at 35.3 mg/kg bw (the lowest tested dose) - In 28 day diet in mice : at 46.7 mg/kg bw (the lowest tested dose) - In 90 day diet in rats : at 16.3 mg/kg - In 90 day diet in dogs : at 10.7 mg/kg o Morphological changes : centrolobular necrosis and fatty change in liver, lymphoid necrosis in thymus and spleen, vacuolization in brain, .. observed in rats (at 13.8 mg/kg bw in a 90 day study and at 81.6 mg/kg bw in a 28 day study) The changes occurred at doses below the cut-off value for classification STOT RE cat.2				

Therefore the criteria are fulfilled for cat.2 and we support this classification.
Dossier Submitter's Response
Thank you for the support.
RAC's response
Noted. The RAC needs to discuss the rationale for STOT RE 2 and possibly R48/22 as well. See the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
09.05.2013	United Kingdom	CHEMTURA CORPORATION	BehalfOfAnOrganisation	13
Comment received				
CHEMTURA PROPOSES THAT THE AVAILABLE HIGHER TIER STUDIES SHOULD BE CONSIDERED IN THE CLASSIFICATION CRITERIA ALONG WITH THE REGULATORY GUIDANCE, PUBLISHED LITERATURE AND THE REVIEW OF 91/414/EEC.				
Dossier Submitter's Response				
In contrast to what is stated in the position paper, Regulation 1272/2008 does *not* state that '*It is also recommended that if results of studies of more than one duration are available then those from the study of longest duration should normally be used*'. With regard to different results from studies with different study durations, the guidance to Regulation 1272/2008 states in 3.9.2.3.2 that '*If there are differences in effects at the GV between studies with different duration then more weight is usually given to studies of a longer duration (28 days or more). This is because animals may not have fully adapted to the exposure in studies of shorter durations and also because longer duration studies tend to include more thorough and extensive investigations (e.g. in terms of detailed pathology and haematological effects etc) which can generally give more substantial information compared to shorter duration studies. If a 90-day as well as a 28- day study are available expert judgement has to be used and not just Haber's rule.*' Thus, *all* studies with a duration of 28 days or longer should be taken into account in an expert judgement, not only the study with the longest duration. We agree that the effects in the 78 week oral study in mice from Ivett (1999b) do not fulfil the criteria for classification according to CLP and that results from this (confidential) study should be included. Nevertheless, effects relevant for classification are observed in 3 different studies (3 species, 28 days in rats and mice and 1 year in dogs). We have clearly indicated why the 28 day studies are relevant for classification and why the (negative) results of the longer studies are not per se in contrast with the 28 day studies. We therefore believe that the proposal to classify bifenazate as STOT RE 2, based on effects on the blood, is appropriate.				
RAC's response				
Noted. The RAC needs to discuss the rationale for STOT RE 2 and possibly R48/22 as well. See the RAC opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	14
Comment received				
Page 30-49: Clear evidence of haemolytic anaemia was observed in rats, mice and dogs and is considered relevant for classification according to CLP Regulation 1272/2008 as STOT RE cat. 2.				

Therefore, the proposal by the dossier submitter NL for classification as STOT RE cat. 2 is supported. However, classification according to Directive 67/548 EEC DSD should be considered additionally.

Dossier Submitter's Response

Thank you for the support for classification according to CLP Regulation 1272/2008.
Since the effects observed are above the dose guidance values for classification according to DSD criteria, classification is not required.

RAC's response

Noted. The RAC needs to discuss the rationale for STOT RE 2 and possibly R48/22 as well. See the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Spain		MemberState	15

Comment received

p. 39 Summary and discussion of repeated dose toxicity
The Spanish CA is in agreement with the proposed classification of Bifenazate as STOT RE 2 - (H373: May cause damage to organs (blood) through prolonged or repeated exposure) according to Regulation (EC) 1272/2008.
The main findings included mortality (observed from ≥ 154.8 mg/kg bw/day in mouse) and haemolytic anaemia (observed at 23.9 mg/kg bw/day in dog):
Mortality was observed in 28-day diet study in mouse (Trutter, 1997b), 2/10 males (20%) and 10/10 females (100%) died at 1000 ppm (154.8 mg/kg bw/day for males). The cut off value for a classification for STOT RE in category 2 under CLP from studies on mice (28 days) is 300 mg/kg bw/d. Therefore, classification is necessary.
Anaemia haemolytic was consistently evident across the species (rats, mice and dogs) in the different studies manifested by haematologic changes (decrease of RCB, Hb and Ht), histopathological changes in one or more organs (liver, spleen and bone marrow), clinical biochemistry (increase of bilirrubin) and urinalysis parameters (presence of bilirubin). However, the effects were only sufficiently severe for classification for STOT RE in the one year dietary study in dog (Goldenthal, 1999). Decrease in haemoglobin ≥ 20% at 23.9 mg/kg bw/d for males was observed. The guidance cut off extrapolated value for a classification for STOT RE in category 2 under CLP from a study on dog (1 year) is 25 mg/kg bw/d. Reductions in Hb above 20% are considered sufficient as a stand-alone criterion for classification as STOT RE 2.
In addition there was evidence of neurotoxicity and clinical signs of hypoxia that support this classification as STOT RE 2:
The clinical signs of hypoxia observed in 28-day dietary study in mouse (Trutter, 1997b), at 154.8 mg/kg bw/day in both sexes were dyspnoea and pale appearance due to haemolytic anaemia.
Neurotoxicity was observed in 28 day dietary study in mouse (Trutter, 1997b), at 154.8 mg/kg bw/day in both sexes. It is characterized by ataxia and/or limited use of front and/or hindlimb(s), hypoactive behaviour, hunched posture, head tilt, partial closure of eyes, tremors, circling and prostration.
R48/22 is not required since the effects observed are above the dose guidance values for classification according to DSD criteria.
It has to be pointed out that the highest doses tested in the 90-day studies (rat, mouse and dog) were below the cut off values for classification according both DSD and CLP criteria. Therefore, it cannot be excluded that more severe effects than observed could occur at higher doses up to the trigger values for classification after repeated exposure.

Dossier Submitter's Response
Thank you for the support
RAC's response
Noted. The RAC needs to discuss the rationale for STOT RE 2 and possibly R48/22 as well. See the RAC opinion.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
10.05.2013	Finland		MemberState	16
Comment received				
We support the proposed classification according to CLP: Aquatic Acute 1; H400, M-factor of 1, Aquatic Chronic 1; H410, M-factor 1 and the classification according to DSD: N,R50/53 with SCLs of Cn≥ 25 % N; R50/53, 2,5% ≤ Cn<25% N; R51/53, 0.25% ≤ Cn < 2.5%; R52/53 for bifenazate. Degradation: We support the conclusions that Bifenazate is not rapidly degradable and not readily biodegradable. The test guidelines for the degradation studies are not mentioned in the CLH report (with the exception of the ready biodegradability test). The guidelines used could be specified.				
Dossier Submitter's Response				
Thank you for your support. Detailed information on the specific guidelines is not possible, since this this is not mentioned in the DAR. The photolysis studies were determined according to an in-house protocol, the aerobic water/sediment system was studied according to EU and OECD guidelines and anaerobic water/sediment system was studied according to an in-house protocol.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.05.2013	Belgium		MemberState	17
Comment received				
Based on the results of the aquatic toxicity test on the most sensitive species (Acute :Skeletonema costatum with 96hEC50 = 0.36 mg/l ; chronic : tests for all three trophic levels, with most sensitive species Oncorhynchusmykiss with 87dNOEC= 0.017mg/l(mm)) and the fact that the substance is considered as not rapidly degradable it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic Acute 1, H400 and Aquatic chronic 1,H410. In view of the proposed classification and toxicity band for acute toxicity between 0.1 and 1 mg/l, an M-factor for acute toxicity of 1 could be assigned, and an M-factor for chronic toxicity of 1 (not rapidly degradable substance and toxicity band between 0.01and 0.1 mg/l).				

Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, bifenazate should be classified as N, R50/53

In conclusion : we agree with the proposed environmental classification.

Some editorial or/and minor comments
Aquatic toxicity :
Please explain how corrections for purity and recovery were performed.

Dossier Submitter's Response

Thank you for your support.

The following information was taken from the DAR to address the issue of purity and recovery corrections for the two key studies:
Skeletonema costatum.
Study material was bifenazate technical (D2341) with purity of 92.6%. Samples were collected at t=0 and 96 hours and analysed for bifenazate and metabolites D3598 and D1989 using HPLC with UV detection. Samples were acidified with phosphoric acid, diluted with methanol and centrifuged. Method limit of quantification was 40 µg/L for bifenazate and metabolites. The mean procedural recovery was 94.5 ± 4.60% (n=6) for bifenazate, 102 ± 6.58% (n=6) for D3598, and 98.6 ± 6.04% for D1989 (n=6).
Actual concentrations of bifenazate in t=0 samples, corrected for purity of the test compound, were 71.7 to 84.1% of nominal and amounted to 0.0452, 0.101, 0.200, 0.420, and 0.815 mg/L. Concentrations at t=96 h were below the LOQ for the lowest and the two highest test concentrations, and were 43 and 46% of nominal at test concentrations of 0.13 and 0.25 mg/L (0.055 and 0.114 mg/L). Concentrations of D3598 and D1989 were below the LOQ in all samples.

Oncorhynchus mykiss
Study material was bifenazate technical (D2341) with purity of 92.6%. Water samples were collected on test initiation and termination and weekly in between. Additional sampling was performed when a sampling or analysis error occurred. Water was analysed for bifenazate and metabolites D3598 and D1989 by HPLC with a tunable absorbance detector. Limit of quantification was 0.01 mg/L. Mean analytical recovery 99.6 ± 5.59% (n=42) for bifenazate, 93.5 ± 10.2% (n=42) for D3598 and 99.2 ± 3.73% (n=42) for D1989.

Average measured concentrations over the whole test period, corrected for purity of the test compound and procedural recovery, were 0.017, 0.037, 0.079, 0.14 and 0.28 mg/L, which represents 68 to 79% of nominal. D3598 was detected in samples of all bifenazate treatments, concentrations expressed as bifenazate equivalents were 11 to 78% of the nominal bifenazate concentrations. Highest proportions of D3598 were found at the lowest test concentration (49-78% of nominal bifenazate, average 57%, 6 out of 28 samples), D3598 reached average amounts equivalent to 17 to 33% of nominal bifenazate in the other treatments. Concentrations of D1989 were always below the LOQ. The mean summed concentrations of bifenazate and D3598, expressed as total bifenazate equivalents, were 0.0192, 0.044, 0.091, 0.163, and 0.31 mg/L. This represents 77 - 91% of nominal.

Actual concentrations of bifenazate <80% of nominal, recovery of total bifenazate equivalents was 71-101%. At the NOEC level (0.025 mg/L nominal), average bifenazate recovery was 70%, recovery of total bifenazate equivalents was 77%. Average concentrations of D3598 at the NOEC-level were equivalent to 33% of the nominal bifenazate level, but D3598 was detected in only 13 out of 28 samples. The lowest NOEC is

0.017 mg/L based on measured bifenazate (0.0192 mg/L based on total equivalents).
RAC's response
The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.05.2013	Spain		MemberState	18

Comment received

We have some comments regarding the Dutch environmental classification proposal. We consider that this substance should be classified based on the aquatic toxicity of the metabolite D3598, since it is more toxic than the parent.

According to the table 5.4-2:

The aquatic acute toxicity of D3598 is:

Oncorhynchusmykiss (flow through) 96h LC50 = 0.044 mg/L
Daphnia magna (flow through) 48h EC50 = 0.051 mg/L
Pseudokirchneriella subcapitata (static) 96h ErC50 > 1.8 mg/L

All measured.

Aquatic long term toxicity of D3598 is:

Only one measured 96h algae NOEC is available and its value is 0.56 mg/L

Incomplete information about the degradability of the D3598 is submitted, an inconclusive hidrolisys test and some data on simulation test (sediment DT50= 10 d). So it could be considered that D3598 is not rapidly degradable. Furthermore, information on log Kow and/or bioaccumulation has not been presented.

Therefore, according to the abovementioned data our classification proposal is:

Under Directive 67/548/EEC:

N R50/53 with SLC of 10
Cn ≥ 2.5% N; R50/53
0.25% ≤ Cn ≤ 2.5% N; R51/53
0.025% ≤ Cn ≤ 0.25% R52/53

Under CLP Regulation

Acute 1, M factor 10
Chronic 1, M factor 10

Dossier Submitter's Response

Thank you for your comment.

Although we agree that based on the results of the aquatic toxicity tests, the primary degradation products of bifenazate are considered classifiable for the environment, we are of the opinion that the environmental classification for

bifenazate should not be based on the data from the degradation products.

In the CLP Guidance, section I.4.1 (c), it is stated that "where the toxicity can be attributed to a degradation breakdown product, andthe concentrations of this are known, the L(E)C50 for classification purposes may becalculated based on the geometric mean of the degradation product concentration,back calculated to the parent substance". In the case of bifenazate,the available analytical information shows that both bifenazate and (more than one) degradation products are present in the test solution. The aquatic toxicity data further show that bifenazate self is toxic to the aquatic environment. Therefore, the observed toxicity is not due to the degradant alone but also to bifenazate. The contribution of the degradants depends on how much of them are formed during the study; the formation of the degradants is not necessarily always constant. We feel that the current approach of representing the data in terms of bifenazate equivalents is most appropriate since it will take the toxicity of the primary degradant for which information is available into account.

RAC's response

Noted. The RAC supports the DS approach to classify bifenazate based on the aquatic toxicity data for bifenazate expressed as bifenazate equivalents, because this approach also takes into account toxicity of primary degradation products.

Date	Country	Organisation	Type of Organisation	Comment number
08.05.2013	Germany		MemberState	19

Comment received

The use of data from tests with salt water organisms as well as fresh water organism tests is usual for classification and labeling purposes for the environment. As a general remark we suggest to use for classification and labeling of acute or chronic effects of the substance the lowest available EC/LC50 or NOEC values.
It would be helpful for clarification to complete the following study results:

Page 68 Short term toxicity to fish and page 65 table 5.4-1
Graves,W.C. and Krueger,H.O. (1999) report 117A-104:
This study was run in accordance with EPA and ASTM guidelines with the sheepshead minnow Cyprinodon variegatus with bifenazate in a flow-through system over a period of 4 days. The LC50 (4d) is 0.42 mg/L based on mean measured bifenazate equivalent (bifenazate and its degradation metabolite D23-06) concentrations.
For classification of the acute risk of bifenazate we suggest to use this lowest LC50 (4d) of 0.42 mg/L (mean measured bifenazate equivalents) for fish.

Page 69 Short term toxicity to aquatic invertebrates and page 66 table 5.4-1
Graves,W.C. and Krueger,H.O. (1999) report 117A-105:
This study was run in accordance with EPA and ASTM guidelines with the saltwater mysid Mysidopsisbahia with bifenazate in a flow-through system over a period of 4 days. The LC50 (4d) is 0.23 mg/L based on mean measured bifenazate equivalent (bifenazate and its degradation metabolite D23-06) concentrations.
For classification of the acute risk of bifenazate we suggest to use this lowest LC50 (4d) of 0.23 mg/L (mean measured bifenazate equivalents) for invertebrates.

Page 70-71 Comparison with criteria for environmental hazards
Please correct the relevant data for acute classification and labeling according the

completed LC50 values of the 2 above mentioned tests.
The acute M-factor of 1 stay the same, because the new relevant values LC50 (4d) of 0.42 mg/L (mean measured bifenazate equivalents) for Cyprinodon variegatus and LC50 (4d) of 0.23 mg/L (mean measured bifenazate equivalents) for Mysidopsisbahia are in the same range as the relevant values cited in the CLH-report for fish Lepomis macrochirus LC50 (4d) of 0.58 mg/L (mean measured) and for invertebrates Crassostrea virginica EC50 (4d) of 0.417 mg/L (mean measured).

Dossier Submitter's Response

Thank you for your comments.
We agree that the use of data from salt water organisms is acceptable for classification and labelling purposes for the environment. As requested, further details for the two short term toxicity studies with salt water organisms are given below. The summaries are taken from the DAR as we do not have the actual study reports.

However, we prefer not using these studies and for classification and labelling because of the uncertainties in the actual concentrations of bifenazate and total bifenazate equivalents in these studies. The RMS for the DAR considered neither study acceptable for risk assessment purposes. When compared to the key studies cited in the CLH report, both of these studies are considered to have more uncertainty (of lower quality) than the studies that are currently used in the CLH report.
As mentioned by the MS the acute classification and M-factor remain the same if these studies are used as the basis for classification. Therefore we have a preference for using the more reliable toxicity values obtained for the fish *Lepomis macrochirus* (LC50=0.58 mg/L) and invertebarate, *Crassostrea virginica*(EC_{50}=0.42 mg/L). However, these studies can be regarded as additional and supporting information for acute toxicity in fish and invertebrates, showing that bifenazate has similar aqutic toxicity in several species.

Study 1: Graves,W.C. and Krueger,H.O. (1999) report 117A-104.

The acute toxicity of bifenazate technical (D2341, purity 92.2%) for the sheepshead minnow was tested in accordance with EPA and ASTM guidelines. Juvenile sheepshead minnows (length 20-29 mm, weight 0.23-0.60 g) were exposed to five nominal concentrations ranging from 0.16 to 1.2 mg/L in two replicates of ten fish each (mean loading 0.26 g/L). A negative and a vehicle control (acetone, 0.1 mL/L) were included in the test. Test media were prepared in natural seawater diluted to a salinity of ca. 20 ‰ (DO values 6.9-7.2 mg/L). Samples collected at t=0, 48 and 96 hours were analysed for bifenazate, and metabolites D3598 and D1989 by HPLC with a tunable absorbance detector.

DO values were within the accepted range. Stock solutions contained 93-98% of the target concentration. A tan/white precipitate was observed on the sides of the mixing chambers, all test solutions in the test chambers appeared to be clear. Actual concentrations of bifenazate, corrected for purity of the test compound and analytical recovery, were on average 26-38% of nominal and amounted to 0.061, 0.098, 0.16, 0.19 and 0.41 mg/L. Metabolite D3598 was detected in all test concentrations, average concentrations were 0.074, 0.11, 0.16, 0.25 and 0.32 mg/L expressed as bifenazate equivalents (not corrected for procedural recovery). Test concentrations, expressed as total equivalents of bifenazate, were 0.14, 0.21, 0.34, 0.44, and 0.73 mg/L which represents 61 to 88% of the nominal bifenazate concentration.D1989 was detected in chromatograms but was below the limit of quantitation.

No mortality was observed in the control groups and the three lowest test concentrations, full mortality occurred at the highest concentration. As the sponsor provided information that toxicity of D3598 is similar to that of bifenazate, the total bifenazate equivalents were

used to estimate LC_{50} and NOEC. An actual 96-hours LC_{50} of 0.42 mg/L (95% CI 0.32-0.73 mg/L) was calculated using the binomial method, based on total bifenazate equivalents. The 96-hours NOEC was 0.32 mg/L as total bifenazate equivalents.

Remark by RMS: Procedural recovery for D3598 is too low. The above mentioned assumed similar toxicity of D3598 originates from screening test in which concentrations were not measured. Actual concentrations of bifenazate were <80% of nominal in all concentrations and total bifenazate equivalents were <80% of nominal in all but the lowest test concentration. The results are not used for risk assessment.

Study 2: Graves,W.C. and Krueger,H.O. (1999) report 117A-105.

The toxicity of bifenazate technical (D2341purity 92.2%) for the mysid shrimp was determined in accordance with EPA and ASTM guidelines. Shrimps were exposed to nominal concentrations of 0.063, 0.13, 0.25, 0.50, and 1.0 mg/L, two replicates per concentration with 10 shrimps each. A negative and a vehicle control (acetone, 0.1 mL/L) were included in the test. Test media were prepared in filtered natural sea water diluted to a salinity of ca. 20 ‰ (DO values 6.7-7.3 mg/L).Samples collected at t=0, 24, 48 and 96 h were analysed for bifenazate, and metabolites D3598 and D1989 by HPLC with a tunable wavelength detector.

DO values were within the accepted range. Stock solutions contained 78-80% of the target concentration. A tan/white precipitate was observed on the sides of the mixing chambers of all but the lowest test concentration, but all solutions in the test chambers appeared to be clear. Actual concentrations of bifenazate, corrected for purity of the test compound and analytical recovery, were on average 14-37% of nominal and amounted to 0.023, 0.043, 0.069, 0.11 and 0.14 mg/L. Metabolite D3598 was detected in all test concentrations, average concentrations were 0.037, 0.069, 0.13, 0.18, and 0.25 mg/L expressed as bifenazate equivalents (not corrected for procedural recovery). This represents 25 to 59% of the nominal bifenazate concentrations. Test concentrations, expressed as total equivalents of bifenazate, were 0.060, 0.11, 0.20, 0.29, and 0.39 mg/L. This represents 95, 85, 80, 58 and 39% of the nominal bifenazate concentration. D1989 was identified in most chromatograms, but concentrations were below the LOQ.

Shrimps in controls and the two lowest test concentrations appeared to be healthy. Mortality after 96 hours was 25% at 0.20 mg/L total bifenazate equivalents and full mortality was reached at the highest test concentration. As the sponsor provided information that toxicity of D3598 is similar to that of bifenazate, total bifenazate equivalents were used to estimate EC_{50} and NOEC. The 96-h EC_{50}, based on total bifenazate equivalents, was calculated as 0.23 mg/L (95% CI 0.21-0.25 mg/L) using Probit analysis. The NOEC was reported as 0.11 mg/L expressed as total bifenazate equivalents.

Remark by RMS: Actual concentrations of bifenazate in the two highest test concentrations <80% of nominal. At the EC_{50} level of 0.29 mg/L, recovery of total bifenazate equivalents is also likely to be <80%. The result is not used for risk assessment.

RAC's response

Noted. The RAC agree with the DS approach that these studies can be regarded as additional and supporting information for acute toxicity in fish and invertebrates.

Attachments:

1. BIFENAZATE: Review of Toxicity end points used for the proposed harmonised classification and labelling (STOT RE CATEGORY 2) (file name: Bifenazate and STOT RE position paper.docx) provided by CHEMTURA CORPORATION on 09/05/2013