Committee for Risk Assessment

RAC

Annex 3

Records

of the targeted public consultation following submission of additional experimental aquatic toxicity studies

hexyl 2-(1-(diethylaminohydroxyphenyl) methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate

EC Number: 443-860-6

CAS Number: 302776-68-7

CLH-O-0000001412-86-253/F

Adopted

30 November 2018

ANNEX 3 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON HEXYL 2-(1-(DIETHYLAMINOHYDROXYPHENYL)METHANOYL)BENZOATE; HEXYL 2-[4-(DIETHYLAMINO)-2-HYDROXYBENZOYL]BENZOATE

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.

ECHA accepts no responsibility or liability for the content of this table.

The proposal for the harmonised classification and labelling (CLH) of (hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate, EC 443-860-6; CAS 302776-68-7) was submitted by Germany and was subject to a public consultation, from 14/11/2017 to 12/01/2018. The comments received by that date are compiled in Annex 2 to the opinion.

However, on 4 September 2018, additional information (experimental studies) was submitted relating to the aquatic toxicity of the substance. A target consultation was launched from 17/09/2018 to 01/10/2018 and the comments received are listed below.

Substance name: hexyl 2-(1-(diethylaminohydroxyphenyl)methanoyl)benzoate; hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate
EC number: 443-860-6
CAS number: 302776-68-7
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
21.09.2018	Germany	BASF SE	Company-Manufacturer	1
Comment received				
Dear ECHA Team, Please find attached the cover letter. This document is the related cover letter, giving an overview of the additional data submitted, their reason and background as well as the summarized outcome and conclusion of the data. Best regards, Kathrin ECHA note – An attachment was submitted with the comment above. Refer to public attachment Cover letter for additional documents (20.09.18).pdf				
Dossier Submitter's Response				
RAC's response				
RAC would like to thank the company for providing an overview of the additional data submitted, their reason and background as well as the summarized outcome and conclusion of the data.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2018	Germany		MemberState	2

Comment received

Summary of the data provided in July 2018 for clarification on aspects of the IBACON (2007) long-term toxicity study on Daphnia magna
Some additional data on the appearance of the test substance in the test media in the chronic daphnia study on which the classification is based (IBACON 2007) is provided. On page 37 it is described that „at all concentrations iron was partially precipitated; swims at the surface". At day 2, 9, 12 "at all concentrations iron is at the flea and at day 7 for the 10 µg/L and 32 µg/L concentration it is noted that iron is partially precipitated".
It is described, that the "iron" precipitation was not quantified. In the chronic daphnia study IBACON (2007) all test solutions were stirred for 2 to 3 days.

Also an analysis report was provided (18A01077), where some additional experiments on the recovery of Cu, Fe, Mn, Mo, Zn from the M4 medium after 2 days with and without stirring/ at room temperature (RT) and 30°C/ with 32 µg Uvinul A Plus/L were conducted. The analytical method (ICP-MS) did not allow to distinguish between different species such as Fe2+ and Fe3+.
There was a difference observed in the Fe-content between the stirred and not stirred solutions (0.06 to 0.12 mg/L).

In a second analysis report (18A01078), an experiment on the influence of stirring speed was conducted (Cu, Fe, Mn, Mo analysis of M4 medium after 2 or 4 days without, with slow, moderate, fast stirring; all at RT).
As a result there were differences between the stirred (0.13 mg/L) and not stirred solutions (0.17 mg/L) with respect to the iron content. For the manganese concentration there was a difference between the fast stirred solution at 4-days of storage and the other solutions (without, slow, and standard stirring).
For both experiments there was no information on replicates and therefore the variation of the iron content within the same solution was not apparent.

The registrant provided an "additional data declaration". Here, also some publications (Dave, 1984; Hudson et al., 2016) were quoted, which describe the behaviour of daphnia in reproduction tests with different Fe2+-contents. Hudson et al. (2016) observed that daphnids started reproduction significantly later, when fed with algae containing lower contents or no Fe2+– 8-9 to roughly 11 days until the start of the reproduction (clear dose-response correlation). They examined the effects of a low iron diet together with a methylmercury exposure. The different iron contents (no, low, high) in the diet did not result in significant effects on time to first reproduction, average brood size or reproduction rate. Therefore, the potential difference in the iron content of test medium is likely to have no consequence for toxicity of the test substance to Daphnia. According to Elendt and Bias (1990), who developed the Elendt M4-medium, the selenium and not the iron concentration is essential for better growth and reproduction of Daphnia. The iron content in algae for the experiment was: 100% (3.16 mg/L), 50% (1.58 mg/L), 10% (3.2 mg/L), or no iron. The second publication cited is (Dave, 1984), where significant effects of the Fe2+-concentration on reproduction rate, i.e. a stimulation of daphnids reproduction at low iron concentrations, were observed. Dave (1984) used the following iron concentrations: 0.0000625, 0.000125, 0.00025, 0.0005, 0.001, 0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256, 0.512, 1.024, 2.048, 4.096, 8.192, and 16.384 mg/L. At 0.512 mg Fe/L and higher a brown precipitate was observed and the survival was lower than in the control

(after 21d). At 0.064 and 0.128 mg/L there were significant differences in progeny per female and survival.
The differences in iron concentration in the experiments mentioned above (first and second analysis report – 18A01077 and 18A01078) were lower than in the publications. The iron content in the test medium (according to the analysis reports) seems to be much lower than in the experiment of (Hudson et al., 2016) or seems to affect daphnia - if at all – in the same way.

Data set provided in September 2018 containing i.a. two new long-term toxicity tests on Daphnia magna
A Daphnia reproduction test according to OECD 211 was conducted. Daphnia were cultured for 21 days in M4-medium excluding the Fe(II)-EDTA-complex. No statistically significant effect on parent mortality, parental length, number of living offspring, number of immobile neonates, aborted eggs and time to first brood over 21-days in daphnids cultured in Fe(II)-EDTA-complex-free medium compared to the control (standard M4- medium; Wilcoxon test, one-sided, not significant). However, daphnids raised in the absence of Fe(II) in the M4-medium demonstrated a delay in the average brood deposition day of the first four broods. Whereas this delay was not statistically significant after the first, second and third brood, the delay of the fourth brood was statistically significant compared to the control group (standard M4-medium; Wilcoxon test, one-sided, $p \leq 0.05$). This can lead to a misinterpretation of the fecundity endpoint if the test is terminated on day 21.

A chronic daphnia study with the test substance was conducted as a limit-test (11.4 µg/L) with Daphnia magna supplied by the Institut für Biologische Analytik und Consulting (ibacon) GmbH (Reference substance sodium chloride 48h-EC50= 4.31 g/L; range 3.88 – 7.22 g/L). For the preparation of a saturated stock solution a saturation column was used. The initial measured concentrations at the start of each renewal interval ranged from 73 to 99 %. The recovery at the end ranged from 36 to 119%. Semi-static test conditions with 16 h light per day, 10 replicates with 1 parent animal each. No significant mortality, reduced reproduction or any other additional significant adverse biological effects or abnormal behavior were observed in any of the test treatments.

Another chronic daphnia study with the test substance was conducted as a limit-test (12.7 µg/L) with the Daphnia magna clone "M10" supplied by ECT Oekotoxikologie GmbH (Reference substance sodium chloride 48h-EC50= 5.08 g/L; range 3.88-7.22 g/L). For the preparation of a saturated stock solution a saturation column was used. The initial measured concentrations at the start of each renewal interval ranged from 68 to 133 %. The recovery at the end ranged from 32 to 108%. The Limit of quantification was 5 µg/L. Semi-static test conditions with 16h light per day, 10 replicates with 1 parent animal each were used. No effects were observed.

Additionally, a water solubility study (08E03159; 06.03.2009) was provided, in which two methods were used: a) column eluate method using M4-Medium or Milli-Q-water and b) flask method using Milli-Q-water. The first method (a) using M4-Medium resulted in a maximum water solubility of 13 ± 6 µg/L. Using Milli-Q-water and the first method resulted in a maximum water solubility of 16 ± 3 µg/L and the second method (b) in a maximum water solubility of 25 µg/L.

Conclusion on the data provided:
There are indications that in the long-term toxicity test on Daphnia magna, which is the

basis of the proposed classification (IBACON 2007), the iron concentration slightly differed between the control and the test solutions with test substance because the control was not stirred in contrast to the other test solutions. In the cited publications no effects in the relevant iron concentration range on Daphnia are reported. One test provided hints that Daphnia showed a delay of the fourth brood but not for the first, second or third brood after exposure to M4-Medium without iron and that this may lead to a misinterpretation of the results if the test is not extended. In the IBACON (2007)-study, the delay of the fourth brood, observed in the study with M4-Medium without iron, was not observed. The derivation of the NOEC for reproduction after the third brood resulted in the same NOEC as after the forth brood.
Taking into account all data provided for the IBACON (2007)-study, the German CA comes to the conclusion that it is possible that the effects occurred due to the particles (physical effect) and not due to the intrinsic properties of the test substance. In the IBACON (2007) study a supersaturated approach was used and undissolved test substance may have been present in the test solutions. Additionally, there are three valid Limit tests showing no effects (Limit-tests at 14.2, 11.4, or 12.7 µg/L).

References:
Dave G. (1984): Effects of waterborne iron on growth, reproduction, survival and haemoglobin in Daphnia magna. Comp Biochem Physiol C 78 (2), 433-438. http://www.ncbi.nlm.nih.gov/pubmed/6149093
Hudson S.L., Doke D.A., and Gohlke J.M. (2016): The effect of a low iron diet and early life methylmercury exposure in Daphnia pulex. Food Chem Toxicol 89, 112-119. DOI: 10.1016/j.fct.2016.01.012

Dossier Submitter's Response

RAC's response

RAC would like to thank you for providing tha assessment of the additional data submitted by industry.

Date	Country	Organisation	Type of Organisation	Comment number
01.10.2018	United Kingdom		MemberState	3

Comment received

Based on the information available in the original Public Consultation and this targeted Public Consultation, we consider that there are uncertainties regarding the BASF, 2007 chronic toxicity to Daphnia magna study endpoints which impact the study reliability.
We agree that three valid chronic toxicity to Daphnia magna studies demonstrate no effects to the limit of solubility in test media.

Dossier Submitter's Response

RAC's response

Thank you for your comment.

Date	Country	Organisation	Type of Organisation	Comment number
26.09.2018	France		MemberState	4
Comment received				
Two new OECD 211 tests provide useful information on the solubility of the substance, corresponding to a maximum mean measured concentration of ± 20 % of the maximum concentration, which can be achieved under test conditions. This provides low NOEC and LOEC value (between 11.4 and 12.7 µg/L) fitting with the criteria set in the CLP for Non-rapidly degradable substances for long-term aquatic chronic toxicity Cat. 1. France agrees with the proposal of changing the classification from Aquatic Chronic 4 H413 to Aquatic Chronic 1 H410. Nevertheless, regarding these new data, France is of the opinion that a M factor of 10 is sufficient according to the CLP.				
Dossier Submitter's Response				
RAC's response				
Thank you for your comment. Based on all available information including information made available in the original Public Consultation and targeted Public Consultation RAC is of the opinion that no classification for chronic aquatic toxicity is warranted. The available information shows no adverse effects of the substance to aquatic organisms (fish, daphnia and algae) at concentrations up to the water solubility limit in all reliable tests. According to Table 4.1.0 of CLP Regulation, Aquatic Chronic 4 classification is assigned to poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility and which are not rapidly degradable and have an experimentally determined BCF ≥ 500 (or, if absent, a log Kow > 4), indicating a potential to bioaccumulate. As the latter is clearly not the case, Uvinul A Plus should no longer be classified as Aquatic Chronic 4. Aquatic Chronic 4 classification is not necessary if in addition to the above criteria other scientific evidence exists showing classification to be unnecessary. Such evidence includes chronic toxicity NOECs > water solubility or > 1 mg/L. In case of Uvinul A Plus the available NOECs are all equal to or greater than the water solubility for all three trophic levels. RAC is of the opinion that no classification for chronic aquatic toxicity is warranted. In conclusion, RAC does not support the Dossier Submitter's proposal to classify the substance as Aquatic Chronic 1 (H410) with M-factor of 1000.				

PUBLIC ATTACHMENTS
1. Cover letter for additional documents (20.09.18).pdf [Please refer to comment No. 1]