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Helsinki, 12 April 2019

Substance name: Climbazole
EC number: 253-775-4
CAS number: 38083-17-9
Date of latest submission(s) considered[1]: 6 May 2015
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressee(s): Registrant(s)[2] of climbazole (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

Based on Article 46(1) of the REACH Regulation (Regulation (EC) No 1907/2006), ECHA requests you to submit the following information on the registered substance[3]:

Fish Sexual Development Test (FSDT), test method OECD 234, using Zebrafish (*Danio rerio*) or Medaka (*Oryzias latipes*).

You have to provide an update of the registration dossier(s) containing the requested information, including robust study summaries and a copy of the full study report including appendices and, where relevant, an update of the chemical safety report by **19 January 2021**.

The reasons of this decision and any further test specifications of the requirements are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has a suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals

Authorised[4] by Christel Schilliger-Musset, Director of Hazard Assessment

[1] This decision is based on the registration dossier(s) at the end of the 12-month evaluation period.

[2] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

[4] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

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Appendix 1: Reasons

Based on the evaluation of all relevant information submitted in the registration dossiers on climbazole and other relevant available information, ECHA concludes that further information is required to enable the evaluating Member State competent authority (MSCA) to complete the evaluation of whether the substance constitutes a risk to the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested.

1. Fish Sexual Development Test, test method OECD 234, using Zebrafish (*Danio rerio*) or Medaka (*Oryzias latipes*)

The concern(s) identified

1. Endocrine disruption (ED) concern from structurally related substances (*in vitro* and *in vivo*)

Climbazole is an imidazole compound. A review (Matthiessen and Weltje, 2015) published in late March 2015 highlighted the endocrine disruption potential of some azoles, including imidazoles, in fish. There is experimental evidence that under laboratory conditions some azoles cause masculinisation or defeminisation in fish by inhibition of the cytochrome P450 enzyme aromatase (CYP19). This aromatase inhibition appears to be the dominant mode of endocrine action in fish and spans a range of potencies for different azoles. In addition, there is evidence that some azoles (e.g. the imidazole compounds ketaconazole and prochloraz) interact with endocrine systems to inhibit testosterone production in fish or block the fish androgen receptor. For example, the endocrine disruption potential of prochloraz is well documented and used as a test example in OECD Guidance Document 181 (OECD, 2012). ECHA concludes that climbazole belongs to a class of chemicals that have the potential to interfere with the endocrine system in fish, but direct read across between substances is not currently possible given the range of potencies. Thus, there is necessity to clarify the ED concern.

2. ED concern from in vitro ToxCast Tox21 assays on the registered and the structurally related substances prochloraz and triadimenol

Tox21 Program carried out in the United States (US EPA, 2015a) includes *in vitro* screening assays for both climbazole and the structurally related substance prochloraz (National Center for Biotechnology Information, 2015).

ECHA has reviewed the Tox21 database for climbazole. The results were as follows:

- The single Aromatase Inhibition (AI) assay was positive.
- All Estrogen Receptor (ER) assays were inactive.
- Three Androgen Receptor (AR) assays were available – one was inconclusive (antagonist) and two were inactive (agonist). ECHA notes that the results for the same Tox21 AR assays for prochloraz (two different tests) were: one active (antagonist), three were inconclusive (antagonist) and two were inactive (agonist). This suggests that the Tox21 human cell-based assays may not be appropriate for assessing AR ecotoxicological effects which is well documented for prochloraz (see Matthiessen and Weltje, 2015).
- One Aryl hydrocarbon Receptor (AhR) assay for climbazole was active.

The Tox21 *in vitro* data reflect a rapid screening, high throughput set of assays. While one ER assay protocol has been validated (although not for high throughput), the remainder are not validated or internationally recognised assays. Therefore, ECHA considers that the Tox21 data described above have a relatively high uncertainty. Nevertheless the data

indicate that climbazole could have several ED modes of action as observed for other imidazoles.

An academic study (Chen et al, 2015) considered AI potential by screening positive AI results from the Tox21 database through an AroER tri-screen assay. This process screens the presence of testosterone and 17β-estradiol to distinguish between AIs and ERα (Estrogen Receptor beta) antagonists. The study identified climbazole as a potential Aromatase Inhibitor (presented in the supplementary information). The main paper also noted that structural activity was not only linked to the 1,2,4-triazole class but other structures including imidazoles also exhibited AI potential. This further confirms a concern for climbazole that needs to be followed up.

ECHA highlights that the *in vitro* data summarised in the prochloraz case study (OECD 2012) cited above, includes positive results in *in vitro* assays including an androgen receptor binding assay performed according to the US EPA OPPTS 890.1150 test guideline. Prochloraz is also one of the 10 substances specified in the Stably Transfected Human Androgen Receptor Transcriptional Activation Assay (OECD TG 458) to demonstrate laboratory proficiency for the (AR) assay.

You have previously highlighted that the substance triadimenol as a potential analogue has been considered by the US Environmental Protection Agency (US EPA) Endocrine Disrupter Screening Program (EDSP). In that programme triadimenol was not considered a priority substance for further endocrine testing (US EPA, 2015b), which you argued was supported by a Tox21 estrogen receptor (ER) assay which was inactive. However, ECHA notes that the EDSP program is based on ER bioactivity using the ToxCastTM Endocrine Receptor Model and that the concern in this case is related to androgen receptor activity and aromatase inhibition, not estrogen receptor activity. In addition, triadimenol was active in a Tox21 AI assay supporting AI potential concern (National Center for Biotechnology Information, 2015).

3. Concerns related to the exposure potential

Climbazole is supplied at a relatively low tonnage. However, it is not readily or inherently biodegradable. According to information in the chemical safety report the substance is used in cosmetics and personal care products, with a wide dispersive use. Given this wide dispersive use pattern and available information on degradation, aquatic exposure is likely. Actual exposure of the aquatic environment is confirmed by a very limited survey of [redacted] rivers using a semi-quantitative screening analytical method ([redacted], 2014) and data in your CSR ([redacted], 2013).

Together, these hazard and exposure data provide a potential concern for environmental endocrine disruption and further information is required to investigate this.

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Why new information is needed

Available information shows that there is a concern for potential endocrine disruption (ED) for the environment, but there is insufficient evidence to clarify this concern. This confirms the need for further investigation in order to conclude on environmental ED potential for climbazole, as information on the mode of action and adverse effects are required

There is also potential environmental exposure to climbazole. It is noted that the registered substance is solely used in cosmetics and personal care products according to Regulation (EC) No 1223/2009 on cosmetic products (Cosmetics Regulation). However, this legislation does not address the potential risks to the environment arising from use of the registered substance in personal care products and cosmetics. The environmental concerns are considered through the application of the REACH Regulation (Recital 5 of the Cosmetics Regulation). Animal testing for all environmental endpoints is permitted, as also explained in ECHA's factsheet[1] on the interface between REACH and Cosmetics Regulations, which was developed jointly with the European Commission.

What is the possible regulatory outcome

ECHA has requested the OECD TG 234 study to investigate the potential endocrine disrupting properties of the substance. Ultimately, if the obtained data are sufficient to confirm the suspected endocrine disruption properties according to the World Health Organisation/International Programme on Chemical Safety working definition, the evaluating MSCA will assess the need for further regulatory risk management in the form of identification of climbazole as a substance of very high concern (SVHC) under Article 57 (f) of REACH.

Considerations on the test method and testing strategy

The requested FSDT (OECD 234) is the appropriate study to address the concerns identified. It is a level 4 study in the OECD conceptual framework (CF) for Testing and Assessment of Endocrine Disrupting Chemicals(OECD, 2018) that can provide information on the mode of action and adverse effects for identifying the substance as an endocrine disrupter. This will therefore address the concerns highlighted by the analogue data and *in vitro* information for climbazole, including aromatase inhibition and anti-androgenicity.

ECHA notes that the guideline states that a minimum of 3 test concentrations should be used, and 5 test concentrations are recommended if the data are to be used for risk assessment.
Therefore, you are advised to perform the test using 5 test concentrations as it will provide a more precise NOEC/LOEC/EC10 value. In the event that the results indicate that the substance would require identification as a substance of very high concern (SVHC) due to endocrine disrupting properties, a precise NOEC/EC10 would be important for risk management purposes.

[1] https://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf

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In your comments you requested to await the results of the ongoing OECD TG 303 simulation test. Specifically you considered that the results may inform on whether the parent or potential metabolite(s) may need to be tested or whether the results of the fish study are needed to refine the environmental risk assessment and therefore could impact the design of the FSDT.

ECHA acknowledges that the results of OECD 303 simulation test may provide you with some further indications to decide on the number of test concentrations in the FSDT.

ECHA considers that results from this OECD TG 303 cannot inform on the need of the FSDT nor the substance to be tested. Specifically there is already a concern based on the available information that the substance has potential ED effects and this can only be clarified by the FSDT. There is no doubt which substance should be tested i.e. the registered substance. This is because based on the data in your registration dossier little abiotic or biotic degradation occurs, so it is clear that there will not be significant formation of a metabolite(s).

To allow you to decide on the number of test concentration in the FSDT, ECHA grants you an additional 6 months, on top of the 15 months usually granted to perform a FSDT.

You shall submit the full study report for this request. Considering the complexity of the case, a complete rationale of test design and interpretation of results and access to all information available in the full study report (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.) are needed. This will allow the evaluating MSCA to fully assess all the provided information, including the statistical analysis, and to efficiently clarify the concerns.

Consideration of alternative approaches

In the initial draft decision, performing *in vitro* tests such as the OECD 456, 458 or OPPTS aromatase assays was proposed by the eMSCA. However, following discussion with other Member States it was concluded that the outcome of the *in vitro* tests will not fully clarify or remove the concern. Furthermore, in your comments you have also expressed similar concerns and a preference for the FSDT over *in vitro* tests. ECHA considers that for this case there is sufficient evidence to move to a more conclusive *in vivo* testing. This is because the proposed *in vitro* tests might not clarify all potential modes of action arising from the available information. On the contrary the FSDT can be confirmative in respect of ED properties.

Another alternative is to perform OECD CF level 3 tests such as OECD 229, 230 or AFSS (OECD GD 148, OECD 2011). However, in this case, none of the OECD CF level 3 tests will sufficiently cover all suspected modes of action, and are therefore not considered to be good alternatives.

Overall, the request for an FSDT is suitable and necessary to obtain information that will clarify whether there is a potential risk from endocrine disruption. More explicitly, of the

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available alternatives it is the least onerous way to obtain information. ECHA notes that there is no experimental study available at this stage that will provide the necessary information to clarify the concern and avoid the need to test vertebrate animals.

Registrant comments on the original Decision request and the Proposals for Amendment

In your comments on the original draft decision requesting two in vitro studies, you proposed an alternative testing strategy. You proposed that an FSDT (OECD TG 234) would be dependent on the outcome of the sewage treatment plant simulation study (OECD TG 303), which is already in progress.

As explained above ECHA has considered your arguments to await the results of the ongoing OECD TG 303 study.

You also note that there is an absence of available monitoring data in other EU countries apart from the UK. ECHA acknowledges that the UK data is very limited. The absence of EU monitoring does not provide confirmation that there is a lack of environmental exposure, only that measurements have not been undertaken. ECHA also notes that in your CSR you provide further evidence that the use of the chemical results in environmental exposure as you cite STP influent, effluent and surface water monitoring data from [redacted] ([redacted], 2013) stating that the results are 'in good accordance with the estimated freshwater concentrations of 0.4 µg/L for the wide-dispersive consumer use of climbazole based on EUSES calculations.' ECHA considers (limited) environmental monitoring data confirming presence in rivers, and a wide dispersive use pattern, is sufficient to justify the need to investigate whether climbazole has the potential to act as an endocrine disrupter in the environment.

You also argue that the OECD TG 303 could indicate that testing of the ED properties might need to be performed on metabolites rather than parent substance. As already explained above, there is no doubt that the parent substance should be tested in the FSDT. Furthermore, ECHA notes that the OECD difficult substances guidance indicates that if a substance half-life exceeds 3 days, testing should be performed on the parent substance. In your CSR you consider that climbazole is *neither readily nor inherently biodegradable according to OECD criteria*. ECHA also notes that chemical analysis from the available aquatic toxicity studies shows that the substance is generally stable (for example in the 21-day Daphnia reproduction test with a static renewal design, measured concentrations were reported to be 94 - 115% of nominal concentrations). Together, this indicates that the parent substance is much more relevant for testing of the ED properties than any metabolite (if formed). ECHA acknowledges that some degradation may occur in the OECD TG 303, but based on the available biodegradation and chemical analysis data, there is no evidence that this will be so significant as to change the focus of the testing of the ED properties (a more important aspect for the OECD TG 303 study for climbazole is whether the partitioning between sludge and aqueous effluent changes from what is currently estimated).

Deadline to submit the requested Information

In your comments you requested additional time to await the results of the ongoing OECD TG 303 simulation test. You noted that these results are to be available in August 2019. ECHA considers as appropriate to grant you an additional 6 months, on top of the 15 months usually granted to perform a FSDT, to allow you to consider the results of simulation study for the deciding on the number of concentrations in the FSDT.

Conclusion

Therefore, based on the substance evaluation and in accordance with Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the substance subject to this decision:

Fish Sexual Development Test (FSDT), test method OECD 234, using Zebrafish (*Danio rerio*) or Medaka (*Oryzias latipes*).

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References

OECD (2018) Revised Guidance Document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption. Series on Testing and Assessment. OECD Publishing, Paris. https://doi.org/10.1787/9789264304741-en

Matthiessen, P., and Weltje, L. (2015) A review of the effects of azole compounds in fish and their possible involvement in masculinisation of wild fish populations.

OECD (2012) Guidance Document (GD) on standardised test guidelines for evaluating chemicals for endocrine disruption: case studies using example chemicals, Series on Testing and Assessment No. 181.

Endocrine Disruptor Screening Program (EDSP) Estrogen Receptor Bioactivity available at http://www2.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-estrogen-receptor-bioactivity

Chen, S. et al (2015) Cell-Based High-Throughput Screening for Aromatase Inhibitors in the Tox21 10K Library. Toxicological Sciences, 147 (2): 446-457

[redacted]

United States Environmental Protection Agency [US EPA] (2015a). Toxicology Testing in the 21st Century (Tox21) information. Available at: http://www.epa.gov/chemical-research/toxicology-testing-21st-century-tox21

National Center for Biotechnology Information. PubChem Substance Database (2015) Tox21 data available at: https://pubchem.ncbi.nlm.nih.gov/substance. Accessed on 17-September 2015.

United States Environmental Protection Agency [US EPA] (2015b). Endocrine Disruptor Screening Program (EDSP). Available at http://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-overview. Accessed 17 September 2015.

[redacted] (2013). Cited in section [redacted] of your CSR, but without a specific reference

OECD (2011) Guidance document on the androgenised femail stickleback screen. Series on Testing and Assessment No. 148.

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to human health / suspected CMR (reproductive toxicity with unusual and severe general toxicity noted) and human exposure (wide dispersive use, consumer use), climbazole CAS No 38083-17-9 (EC No253-775-4) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The updated CoRAP was published on the ECHA website on 26 March 2014. The competent authority of the United Kingdom (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding worker exposure and, for the environment, the risk assessment and endocrine disruption.

Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information on environmental endocrine disruption.

Following receipt of the worker exposure information requested in the first decision (SEV-D-2114340660-58-01/F), the evaluating MSCA will establish whether there is a need pursue further reproductive toxicity testing to assess the risks from exposure to workers.

The decision making followed the procedure of Articles 50 and 52 of the REACH Regulation as described below.

ECHA notified you of the draft decision and invited you to provide comments.

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took the comments from you, which were sent within the commenting period, into account and they are reflected in the reasons (Appendix 1). The requests were not amended but the deadline was amended.

Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposals for amendment to the draft decision and modified the draft decision by replacing the *in vitro* tests with an FSDT. They are reflected in the reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendments.

Your comments on the proposed amendments were taken into account by the Member State Committee.

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-63 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

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Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to otherwise fulfil the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used ('test material') has to have a composition that is within the specifications of the substance composition that are given by all registrant(s). It is the responsibility of all the registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on the composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.