~~Confidential~~

Helsinki, 02 May 2022

Addressees
Registrant of CAS 63-05-8 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
15/10/2020

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Androst-4-ene-3,17-dione
EC number: 200-554-5
CAS number: 63-05-8

Decision number: Please refer to the REACH-IT message which delivered this communication (in format TPE-D-XXXXXXXXXX-XX-XX/F)

DECISION ON TESTING PROPOSAL(S)

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***9 November 2023***.

The requested information must be generated using the Substance unless otherwise specified.

A. Information required from the Registrants subject to Annex IX of REACH

1. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211)

2. Fish Sexual Development Test (Annex IX, Section 9.1.6., column 2; test method: OECD TG 234) using the Japanese Medaka (Oryzias latipes) or zebrafish (Danio rerio) and including examination of histopathology of the gonads. Five test concentrations must be used as specified in paragraph 30 of the OECD TG 234.

Reasons for the request(s) are explained in the appendix entitled "Reasons to request information required under Annexes IX of REACH".

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH, the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

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 suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Approved[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

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

Appendix A: Reasons to request information required under Annex IX of REACH

This decision is based on the examination of the testing proposals you submitted.

1. Long-term toxicity testing on aquatic invertebrates

Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

Under Article 40(3)(c) of REACH, ECHA may require a registrant to carry out one or more additional tests in case of non-compliance of the testing proposal with Annexes IX, X or XI of the REACH Regulation. The information requirement on Aquatic toxicity at Annex IX covers both long-term toxicity on invertebrates (Section 9.1.5.) and on fish (Section 9.1.6.). However, you have provided a testing proposal for long-term testing on fish only. In case of data gap for long-term toxicity testing on aquatic invertebrates, it is necessary to request this information as an additional test to ensure compliance with the endpoint.

1.1. Information provided to fulfil the information requirement

Your registration dossier does not include any information on long-term toxicity on aquatic invertebrates. Instead, you have provided the following justification to omit the study which you consider to be based on Annex IX, Section 9.1., Column 2:

- *"According to column 2 of REACH Annex IX, long-term testing shall be proposed by the registrant if the Chemical Safety Assessment indicates the need to investigate further the effects on aquatic organisms"*
- *"As the chemical safety assessment resulted in a PEC/PNEC-ratio below 1, the risk towards aquatic organisms is sufficiently controlled based on the available data and no chronic tests are required."*

Furthermore, in the comments to the draft decision, you disagree to perform the long-term toxicity test on aquatic invertebrates, and you mention the following:

- You consider that invertebrates are not particularly sensitive to vertebrate steroid hormones;
- You consider that there is no indication that invertebrates are the most sensitive taxon based on the available acute toxicity data;
- You consider that there is no indication of environmental risk because all the Risk Characterisation Ratios (RCRs) are well below 1 for all identified uses of the substance throughout its life cycle. You indicate your intention to update the environmental risk assessment of the Substance, to revise the PNEC derivations and re-calculate the RCRs if necessary, taking into account the outcome of the OECD 234 study.

We have assessed this information and identified the following issues:

Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to invertebrates. It must be understood as a trigger for providing further information on long-term toxicity to invertebrates further to the information listed in Column 1 of Section 9.1.5. of Annex IX if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

With reference to your comments on the draft decision, ECHA notes that you do not relate these observations to any legal ground for adaptation under Annex XI to REACH. These observations may relate to aspects of your chemical safety assessment. But, as already stated above, the adaptation rule under Annex IX, Section 9.1., Column 2 relates to investigations

of further effects than those investigated by the standard information required under Column 1 of Section 9.1.5. of Annex IX. It cannot serve as a basis to omit the standard information.

Therefore, based on the above considerations, your adaptation is rejected.

1.2. Test selection and study specifications

The *Daphnia magna* reproduction test (test method: EU C.20/OECD TG 211) is appropriate to cover the information requirement for long-term toxicity on aquatic invertebrates (ECHA Guidance R.7.8.4.1.).

1.3. Outcome

Under Article 40(3)(c) of REACH, you are requested to carry out the additional test with the Substance, as specified above.

2. Fish Sexual Development Test

Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.). Further studies than those listed in Column 1 of Section 9.1.6. of Annex IX must be proposed if the chemical safety assessment (CSA) according to Annex I indicates the need to investigate further the effects on aquatic organisms (Annex IX, Section 9.1., Column 2).

2.1 Information is needed to fulfil the information requirement

You have submitted a testing proposal for a Fish Sexual Development test (test method: OECD TG 234). In support of your testing proposal, you have provided the following justifications:

i. no GLP or non-GLP studies are available which cover the endpoint "long-term toxicity to fish" for the Substance
ii. short term toxicity studies and ecotoxicological studies indicate an endocrine mode of action towards aquatic organisms
iii. the Substance is thought to have an endocrine mode of action and could thus cause toxicity to fish as well as disturbance in their sexual maturation, therefore the OECD 234 is proposed.
iv. the conduct of the OECD 234 toxicity test helps to reduce the number of used fish by approximately 50% compared to OECD 240, while providing a similar level of information, although no reproductive phase is included in OECD 234. However, since estrogens or androgens are known to affect the sexual development of young fish, you considered that this test protocol is adequate for the purpose of detecting chronic effects of the Substance as an endocrine active compound.

ECHA requested your considerations for alternative methods to fulfil the information requirement for long-term toxicity on fish. You provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA agrees that an appropriate study on long-term toxicity on fish is needed.

2.2 Test selection

According to IPCS/WHO[2], *"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations".* Based on this definition, data on endocrine activity and adversity of the Substance is needed to assess whether the following conditions are met:

- it shows adverse effects(s) in (an intact) organism, or its progeny, or (sub)populations which include, among others, change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;
- it shows endocrine activity, *i.e.* it has the potential to alter the function(s) of the endocrine system;
- there is a biologically plausible link between the adverse effects and the endocrine activity, *i.e.* the Substance has an endocrine disrupting mode of action (ED MoA).

Under IUCLID, section 7.8, you indicated that the Substance is an endogenous sexual steroid produced by females and males, which is an intermediate in estrone and testosterone biosynthesis and can be metabolized into estradiol.

On this basis, there are indications that the Substance may show endocrine activity by targeting hormone biosynthesis. However, no information is available whether or not the Substance may show adverse effects in aquatic organisms in relation its endocrine activity and therefore whether it has endocrine disrupting properties.

Therefore, ECHA agrees that the chemical safety assessment (CSA) indicates the need for further long-term toxicity test on aquatic organisms than one of the three studies listed in Column 1 of Section 9.1.6. of Annex IX, as none of the latter studies addresses the ED-mediated adverse effects.

The proposed Fish Sexual Development test (test method: OECD TG 234) is an *in vivo* assay (OECD Conceptual Framework Level 4) providing apical information on phenotypic sex ratio which is fixed during fry or juvenile stages of the species used in this test. ECHA considers the proposed test as appropriate to cover the information requirement for long-term toxicity in fish as it informs on ED-mediated adverse effects of substances suspected to impair sexual maturation, such as the Substance.

In your comments to the draft decision, you agree to perform an OECD TG 234 study on the Substance.

2.3 Study specifications

As explained in the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, the assessment of gonad histopathology (e.g. staging of gonads, severity of intersex) is needed for investigating estrogenic, androgenic and steroidogenic (EAS) modalities as it may inform on adversity.

In your comments on the draft decision, you agree to conduct gonad histopathology. However, you consider that the test should be performed on Zebrafish (*Danio rerio*) instead of Japanese medaka (*Oryzias latipes*) as originally requested by ECHA. In support of your comment, you provide the following reasons:

[2] WHO/IPCS, 2002. Global assessment of the state-of-the-science of endocrine disruptors. https://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/.

i) You mention that there is a high variability in hatching for medaka fish which impairs the assessment of the parameters foreseen to be investigated in the OECD 234;
ii) You consider that, although zebrafish do not show primary sexual characteristics, the sex of individual fish can be determined through gonad histopathology.

ECHA has assessed this information and changed the requirement to conduct the study on the Japanese medaka or the Zebrafish. However, ECHA maintains that the preferred species is the Japanese medaka because, although it is acknowledged that sex of individual fish can be determined through gonad histopathology in either Zebrafish or Medaka, gonad histopathology may provide equivocal results in case of occurrence of intersex (i.e. mixed female and male gonads within the same individual). The determination of the genetic sex in Medaka fish enables the unambiguous detection of individual phenotypic sex reversal and, in addition, increases the power of the sex ratio.

In addition, ECHA cannot make an assessment about the impact of the high variability in hatchability on the assessment of the parameters investigated in the OECD 234 as you do not provide specific evidence to document your statement. ECHA notes that the OECD 234 has been validated for Medaka fish, which indicates that this species is adequate to investigate the parameters foreseen to be investigated in the OECD 234.

Based on the above, ECHA concludes that the test should be conducted on the Japanese medaka (*Oryzias latipes*) or the zebrafish (*Danio rerio*). If *Oryzias latipes* is chosen, the requested test must also include genetic sex determination to increase the statistical power of the test result on sex ratio. You must also report any change in the secondary sexual characteristics. As the test is to be used for hazard and risk assessment, it must not be conducted on stickleback because the validation data available so far showed that in this species the alterations of phenotypic sex ratio were uncommon (OECD TG 234).

Adequate information on long-term toxicity to fish is also needed for the purpose of the risk assessment. As specified in OECD GD 150, the OECD TG 234 may also support an evaluation whether specific endocrine-mediated effects may be influenced by general toxicity. In such case, the concentration range needs to be adjusted in order to investigate potential endocrine disrupting effects of the Substance (in the absence of significant non-endocrine mediated effects) and in the same study to investigate other apical endpoints that should be measured including hatching rate, survival, length and body weight. Therefore, to minimize vertebrate testing and to avoid the need to conduct additionally a Fish, Early-Life Stage (FELS) Toxicity Test (test method: OECD TG 210), you must conduct the test with five test concentrations as specified in paragraph 30 of the OECD TG 234.In your comments on the draft decision, you agreed to conduct the test with five test concentrations.

2.4 Outcome

Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test with the Substance, as specified above.

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

1. Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

2. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[3].

B. Test material

1. Selection of the Test material(s)

   The Test material used to generate the new data must be selected taking into account the following:
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test material must contain that constituent/ impurity.

2. Information on the Test material needed in the updated dossier
   - You must report the composition of the Test material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test material is relevant for the Substance.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[4].

[3] https://echa.europa.eu/practical-guides
[4] https://echa.europa.eu/manuals

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 20 November 2020.

ECHA held a third party consultation for the testing proposal(s) from 21 January 2021 until 8 March 2021. ECHA did not receive information from third parties.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

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

ECHA took into account your comments and amended the request.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix D: List of references - ECHA Guidance[5] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[6]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)[7]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[7] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

OECD Guidance documents[8]
Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

[8] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.