~~Confidential~~

Helsinki, 23 November 2021

Addressees
Registrants of JS_72869-86-4 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
22/06/2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate
EC number: 276-957-5
CAS number: 72869-86-4

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 ***28 February 2024***.

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

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

1. *In vitro* micronucleus study (test method: OECD TG 487);

2. Only if **positive** results in the *in vitro* test requested under A.1 are obtained: *In vivo* genetic toxicity study (Annex VIII, Section 8.4., column 2)

   In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

   OR

   In vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in mice or rats, oral route.

The reasons for the request(s) are explained in the following appendix entitled "Reasons to request information required under Annexes VIII 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 and VIII to REACH, for registration at 10-100 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 Christel Schilliger-Musset, 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 VIII of REACH

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

1. In vitro micronucleus study

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is an information requirement under Annex VIII to REACH (Section 8.4.2.).

Further, ECHA guidance R.7a, section R.7.7.6.3 (p.568) specifies that "*In order to ensure the necessary minimum level of information is provided, at least one further test is required in addition to the gene mutation test in bacteria. This should be an in vitro mammalian cell test capable of detecting both structural and numerical chromosome aberrations.*" It is necessary to request an *in vitro* cytogenicity test as an additional test to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy. The obtained *in vitro* data will inform on the genotoxic concern(s) associated with the substance and help identify the most adequate follow-up *in vivo* study.

1.1. Information provided to fulfil the information requirement

You have submitted a testing proposal for an *in vivo* mammalian erythrocyte micronucleus test to be performed with the Substance to further investigate the mutagenicity of the substance.

Your dossier contains an *in vitro* mammalian cell micronucleus test (Schweikl *et al*., 2001) which gave ambiguous results. When the Substance was tested without metabolic activation there was a slight increase in the numbers of micronuclei at cytotoxic concentration levels. You considered that the "*positive result in the in vitro micronucleus test without metabolic activation*" should be followed up by an *in vivo* study "*to conclude on genotoxic potential of the test substance*".

However, in your comments on the draft decision you clarified that the *in vitro* study (Schweikl *et al*., 2001) is an "*old study which did not follow test guidelines*" and "*showed ambiguous results with slight increases in micronuclei at cytotoxic concentrations*". You therefore proposed a step-by-step testing strategy starting with the conduct of a new *in vitro* micronucleus assay according to the OECD test, "*for ethical reasons (limit the use of animals) and to enable a robust basis to decide which in vivo test is relevant*".

ECHA agrees with your assessment that the results of the study results of Schweikl *et al*., 2001 could indicate a concern for chromosomal aberrations. However, we also agree that the study with ambiguous results has some deficiencies, as explained in the following.

To be considered compliant the *in vitro* micronucleus test has to be performed in accordance with OECD TG 487. The key parameters of this test guideline include:

a) The assessment of two separate test conditions: in absence of metabolic activation and in presence of metabolic activation.
b) Treatment schedules: Short term treatment with and without metabolic activation and long-term treatment without metabolic activation.

The reported data for the study you have provided did not include:

a) Two separate test conditions, but only in absence of metabolic activation.
b) The evaluation of the experimental conditions (as explained in OECD TG 487, para. 38), as only the long treatment was performed.

The information provided does not cover the specifications/conditions required by OECD TG 487.

ECHA therefore considers that an appropriate *in vitro* cytogenicity or micronucleus study is necessary to first determine if there is a concern for chromosomal aberration *in vitro*. In case of a positive result, an *in vivo* follow-up study must be performed, as explained under Appendix A.2. below.

1.2 Test design

Either the *in vitro* cytogenicity study in mammalian cells (test method OECD TG 473) or the *in vitro* micronucleus study (test method OECD TG 487) are considered suitable.

In your comments on the draft decision, you propose to conduct the *in vitro* micronucleus study (test method OECD TG 487) to verify the previous *in vitro* results and therefore '*provide a better basis for any in vivo testing strategy*'. ECHA agrees with your considerations; therefore, you should perform the study according to OECD TG 487.

1.3 Outcome

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

2. In vivo genetic toxicity study (conditional testing request)

Under Annex VIII Section 8.4., column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

2.1 Information provided to fulfil the information requirement

You have submitted a testing proposal for an *In vivo* mammalian erythrocyte micronucleus test to be performed with the Substance.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity *in vivo*. 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.

As explained above, under Appendix A, section 1, the *in vitro* mammalian cell micronucleus test (Schweikl *et al.*, 2001) provided is not adequate. Therefore, by this decision, ECHA requests an *in vitro* micronucleus test which may raise a concern for chromosomal aberration in case of positive results.

In case there is a concern for chromosomal aberration, an appropriate *in vivo* follow up genotoxicity study would be necessary to address the concern identified *in vitro.*

Therefore, you must wait for the results of the *in vitro* test requested under A.1. and, only if the test results of request A.1 are positive, to conduct the *in vivo* study as specified below. The deadline set in this decision allows for sequential testing.

2.2 Test selection

According to the ECHA Guidance R.7a, Section R.7.7.6.3, the mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) is generally suitable to follow up a positive *in vitro* result on chromosomal aberration, if the Substance or its metabolite(s) will reach the target tissue. Alternatively, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a suitable test to be performed.

ECHA however notes that, if the genotoxic effect is only observed without metabolic activation, that would mean that the genotoxic effect is due to the parent compound (and not to the metabolites). In such a case the potential effect of the parent (non-metabolised) substance on target tissue(s) can only be detected in the comet assay, as site of contact tissues are analysed in this assay. On the contrary, a test following OECD TG 474, may not detect the effect of the parent substance as it cannot be ruled out that only the metabolite(s) reaches the bone marrow (i.e. the target organ of these tests).

Therefore, as you also indicate in your comments on the draft decision, in case the test results of request A.1 are positive only without metabolic activation you must perform the comet assay and not the MN test.

2.3 Specification of the study design

a) Comet assay

According to the test method OECD TG 489, the test must be performed in rats.

Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

In your comments on the draft decision you indicate that if you perform the comet assay you will perform the test in the following tissues: stomach and duodenum. ECHA notes however, that in line with the test method OECD TG 489, the test must be performed by analysing the default tissues, and these include the liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Germ cells
You may consider to collect the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

b) MN test

You proposed testing in the rat. According to the test method OECD TG 474, the test must be performed in mice or rats.

You proposed testing by the oral route. Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

Regarding the exposure of the target tissue, the applicable test guideline OECD TG 474 states "*If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test*". Additionally, a negative test result can be considered reliable if "*Bone marrow exposure to the test substance(s) occurred*". Accordingly, if the Substance is negative in this test, but it is not possible to demonstrate that bone marrow exposure to the Substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the Substance and whether to request any further information.

2.4 Outcome

Under Article 40(3)(b) your testing proposal is accepted under modified conditions 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[2].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test material) which must be relevant for all the registrants of the Substance.

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 variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity/ additive on the test results for the endpoint to be assessed. For example, if a constituent/ impurity/ additive 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 and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[3].

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

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 19 August 2020.

ECHA held a third party consultation for the testing proposal(s) from 19 October 2020 until 3 December 2020. 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(s).

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[4] 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)[5]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)[6]

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.

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

OECD Guidance documents[7]
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.

[7] 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]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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.