EUROPEAN CHEMICALS AGENCY

Helsinki, 13 January 2020

Addressees
Registrants of [redacted] listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
28/05/2013

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: (1R,5S)-2-(6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl) ethyl acetate
EC number: 800-940-9
CAS number: 35836-72-7

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

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***20 April 2022.***

A. Requirements applicable to all the Registrants subject to Annex IX of REACH

1. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum, with the Substance;

2. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method OECD TG 408) in rats, with the Substance;

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method OECD TG 414) in a first species (rat or rabbit), oral route with the Substance.

Conditions to comply with the requested information

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.
To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if you have registered a substance at 100-1000 tpa;

Registrants are only required to share the costs of information that they must submit to fulfil the information requirements for their registration.

The Appendices state the reasons for the requests for information to fulfil the requirements set out respectively in the respective Annexes of REACH.

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of he test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

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 for the requests to comply with Annex IX of REACH

In accordance with Articles 10(a) and 12(1) of REACH, a technical dossier registered at 100 to 1000 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII-IX to REACH.

1. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2)

Under Annex IX to 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 and there are no appropriate results already available from an in vivo somatic cell genotoxicity study.

You have provided the following studies in your dossier:
i. an OECD TG 473 study with the Substance,
ii. an OECD TG 487 study with the Substance

We have assessed this information and identified the following issue(s).

The ECHA guidance R.7a[2] states that following a positive result in an in vitro test, *"adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary."*.

Your dossier contains positive results for *in vitro* cytogenicity tests (CA), which raise the concern for chromosomal aberration. However no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

Indeed, the *in vitro* chromosome (CA vitro) aberration study is considered as clearly positive, because of the increases of aberrations observed after 20h exposure without S9 at the concentrations of 60 and 75 µg/mL.

In your comments on the draft decision you have indicated that you do not *"agree with the opinion of ECHA that the results of the study fulfil all three criteria defined in OECD 473 for a positive response. One of the criteria is the demonstration of a dose-related trend using appropriate trend statistics, but the increase was not dose-related when evaluated with an appropriate trend test."*

Furthermore, in your comments to the draft decision you have considered that *"the original evaluation of the slides was not reproducible, when scored by a second peer review slide evaluator."*

ECHA notes that according to the data in the dossier assessed for the draft decision notified to you, the percentages of the cells with chromosome aberrations were 0.5, 3.5 and 5.5 for the three concentrations of 40, 60 and 75 mg/mL, respectively. Therefore, these data

[2] ECHA Guidance R.7a, section R.7.7.6.3, p.570.

indicate that the increase in the percentages is dose-related, and provide an appropriate trend test.

Consequently, the study fulfils all three criteria defined in OECD TG 473 for a positive result, i.e.

a) *"at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control,*
b) *the increase is dose-related when evaluated with an appropriate trend test,*
c) *any of the results are outside the distribution of the historical negative control data (e.g. Poisson based 95% control limits; see paragraph 39)".*

In regard of the results of the second evaluation (concerning the dose-response), the information your refer to in your comments is not in the robust study summary and the circumstances of the second review that may have an impact on its relevance (e.g., whether done only after finalisation of the study report) were not provided. Therefore, the second review cannot be taken into account.

In addition, you have provided an *in vitro* micronucleus (MN vitro) test (OECD TG 487) conducted according to only one of the three usual experimental conditions recommended by the OECD TG 487 (i.e. 24 h treatment, without recovery and without S9). In the CA *in vitro* and the MN *in vitro*, the test substance induced different levels of cytotoxicity. Consequently, the concentrations that caused clastogenicity in the CA *in vitro* test, i.e. 60 and 75 µg/mL, were not analysed in the MN *in vitro* test.

Because of this concern on the test concentrations, ECHA considers that the results obtained in the MN *in vitro* study cannot be used to disregard the positive results obtained in the CA *in vitro* study.

You claim in your comments to the draft decision that "*the micronucleus test was clearly negative up to cytotoxic concentrations, which confirms that the equivocal effects recorded in the OECD 473 study were artefacts and not true aberrations.*"

ECHA considers that the requirement is triggered since there is a positive result in an *in vitro* test. Further, while a positive result in such *in vitro* MN test would confirm that the substance induces chromosomal aberration (as indicated by a positive result of an *in vitro* CA test), the negative result obtained in this MN cannot rule out the result obtained in the CA *in vitro* test, because the concentrations studied do not overlap. In ECHA's view, the fact that the cytotoxicity was higher in the MN did prevent the investigation of the genotoxicity of the substance at doses where it apparently induced chromosomal aberrations in the CA test. Moreover, the difference in cytotoxicity observed in the MN and the CA tests may be due to several issues, e.g. different sensitivities of the human lymphocytes collected from different volunteers, different cytotoxicity calculation methods for the two tests (Mitotic index for the CA and Replication index for the MN), different duration of exposure to the substance (20h in the CA test, 24h in the MN test).

As regards to your comments to the draft decision, in summary, ECHA maintains that 1) the data provided in the robust study summary for the *in vitro* CA test show a positive result for the 20h without metabolic activation, and 2) the negative result obtained in the MN test cannot rule out the result obtained in the CA test, because the concentrations studied do not overlap. Therefore, the results of the chromosome aberration test OECD TG 487 with the Substance is considered positive and the information request is still valid.

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified in vitro.

According to the ECHA Guidance Chapter R.7a[3], the mammalian erythrocyte micronucleus test (OECD TG 474) or the mammalian bone marrow chromosomal aberration test (OECD TG 475) are 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 (OECD TG 489) is a suitable test to be performed.

The genotoxic effect observed in the two *in vitro* tests specified above is observed only without metabolic activation, which means that the genotoxic effect is due to the parent compound (and not to the metabolites). *In vivo*, the potential effect of the parent (non-metabolised) substance on target tissue(s) can be detected in the comet assay, as site of contact tissues are analysed in this assay. On the other hand, the other two *in vivo* tests, i.e. OECD TG 474 and 475, 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, the *in vivo* comet assay is the most appropriate follow-up test for the Substance.

According to the test method OECD TG 489, the test shall 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 line with the test method OECD TG 489, the test shall be performed by analysing tissues from 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

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

> Therefore, you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[4]) in addition to the other aforementioned tissues, 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 accordance to Annex IX, Section 8.4., column 2, 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.

[3] ECHA Guidance Chapter R.7a, Section R.7.7.6.3
[4] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

2. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.)

A Sub-chronic toxicity study (90 day) is a standard information requirement in Annex IX to REACH.

You have provided the following for this endpoint in your dossier:

i. An OECD TG 422 study with the Substance
ii. Data adaptation indicating the treatment related effects observed in the OECD TG 422 study were adaptive, and that no specific target organ toxicity was identified.

We have assessed this information and identified the following deficiencies:

A. OECD TG 422 study

To be considered compliant and enable concluding whether the Substance has dangerous properties and supports the determination of the No-Observed Adverse Effect Level (NOAEL), a study has to meet the requirements of OECD TG 408.

The Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) you have submitted does not have the required exposure duration of 90 days as required in OECD TG 408, because the exposure duration of this screening test was approximately 63 days (for females) and 42 days (for males). Furthermore the organ weight and histopathological investigations in OECD TG 422 are only conducted using 5 animals per sex per group and not 10 per sex per group as in OECD TG 408.

In your comments to the draft decision, you agree to prform the test.

Based on the above, the information you provided do not fulfil the information requirement.

B. Adaptation

You have not provided any legal reference to base your adaptation on and the information provided is not sufficient to fulfil the data requirements of Annex IX, 8.6.2 (see above). Furthermore, we find that there is no adaptation rule in REACH that corresponds to the adaptation you propose.

Therefore, your adaptation is rejected.

Information on the design of the study to be performed (route/ species/ strain)

Following the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity[5]. As the substance is a liquid the sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and with oral administration of the Substance, because the Substance is of very low vapour pressure. Uses with spray application are reported in the chemical safety report. However, the reported concentrations are low [redacted]

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in one species

[5] ECHA Guidance R.7a, Section R.7.5.4.3.

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

In your adapatation you have referred to

i. An OECD TG 422 study with the Substance
ii. Data adaptation indicating that no signs of toxicity to reproduction or on the offspring attributable to test substance were identified in the OECD TG 422 screening test.

We have assessed this information and identified the following issue(s):

A. OECD TG 422 study

In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, information provided has to meet the requirements of OECD TG 414 in one species.

You have not provided information following OECD TG 414. Instead, you have provided a "reproduction/ developmental toxicity screening test" (OECD TG 421)/ "combined repeated dose toxicity study with the reproduction/developmental toxicity screening test" (OECD TG 422). In this study, structural malformations and variations are not investigated as required in the PNDT study (OECD TG 414).

B. Adaptation

ECHA points out that any adaption needs to be based on provisions of REACH. In this case you have not provided any legal reference to base you adaptation on. ECHA notes that the information the waiver is based on is not sufficient to fulfil the data requirements of Annex IX, 8.7. Furthermore, we find that there is no adaptation rule in REACH that corresponds the adaptation you propose.

Therefore, your adaptation is rejected.

In your comments to the draft decision, you agree to prform the test.

Based on the above, the information you provided do not fulfil the information requirement.

A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species with oral[6] administration of the Substance.

[6] ECHA Guidance R.7a, Section R.7.6.2.3.2.

Appendix B: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The compliance check was initiated on 14 January 2019.

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

ECHA took into account your comments and did not amend the requests.

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 C: Observations and technical guidance

1. This compliance check decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of the Member States.

3. Test guidelines, GLP requirements and reporting

   According to Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate, primarily the Test Guidelines approved by OECD as referenced above.

   According to 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.

   According to 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: 'How to report robust study summaries[7]'.

4. Test material

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/impurity is known to have or could have 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.

   Technical reporting of the test material

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

   Technical instructions are available in the manual "How to prepare registration and PPORD dossiers" on the ECHA website (https://echa.europa.eu/manuals).

[7] https://echa.europa.eu/practical-guides

5. List of references of the ECHA Guidance and other guidance/ reference documents[8]

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 in this decision.

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 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)[9]

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.

OECD Guidance documents[10]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.

Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

[8] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[9] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[10] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix D: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.