~~Confidential~~

Helsinki, 22 July 2021

Addressees
Registrants of 265-148-2/64742-46-7 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
08/04/2019

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Distillates (petroleum), hydrotreated middle
EC number: 265-148-2
CAS number: 64742-46-7

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

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by **02 May 2023**.

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. Same In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test (triggered by Annex I, Section 0.5 in conjunction with Annex VIII, Section 8.4., column 2), as requested below in B.1;

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

1. In vivo mammalian alkaline comet assay (test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route (triggered by Annex I, Section 0.5 in conjunction with Annex IX, Section 8.4., column 2). For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

Reasons for the request(s) are explained in the appendices entitled "Reasons to request information required under Annex VIII and IX of REACH", respectively.

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 10-100tpa;
- the information specified in Annexes VII to IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

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

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

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". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations 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 vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test**

Under Annex VIII, Section 8.4, column 2 of REACH, appropriate *in vivo* mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII.

You have provided *in vitro* studies on analogue substances and on the Substance with negative results, except two with an ambiguous result.

However, you conclude that *in vitro* studies are not appropriate to address the mutagenicity properties of the Substance due to the difficulties with its solubility, and that *in vivo* studies are necessary to confirm the intrinsic properties of the Substance.

For that same reason, ECHA agrees that further information is necessary for producing the chemical safety report and that this information can only be obtained by performing an *in vivo* mutagenicity study in accordance with Annex IX to REACH (Section 0.5, Annex I to REACH), to address gene mutation and chromosomal aberration.

For the specifications of the study to be performed, see the request B.1.

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

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

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

Under Annex IX, Section 8.4, column 2 of REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

You have provided (1) *in vitro* studies on analogue substances and on the Substance with negative results, except two with an ambiguous result, and (2) one *in vivo* study on an analogue substance belonging to the OtherGO category.

Your dossier contains key and supporting studies:

i. One key study performed according to a modified procedure of the Ames test (OECD TG 471) with the Substance and which shows a negative result; several other (7) supporting *in vitro* studies performed according to (a modified procedure of) the Ames test (OECD TG 471) with either the Substance, or the analogue substance (Distillates (petroleum), hydrodesulfurized middle (EC number 265-183-3, CAS RN 64742-80-9) or other analogue substances;
ii. One key *in vitro* sister chromatid Exchange (SCE) test (OECD TG 479) with an analogue substance (Distillates (petroleum), hydrodesulfurized middle (EC number 265-183-3, CAS RN 64742-80-9) and which shows an ambiguous result;
iii. One key *in Vitro* Mammalian Cell Gene Mutation Test (OECD TG 476) with the analogue substance (Distillates (petroleum), hydrodesulfurized middle (EC number 265-183-3, CAS RN 64742-80-9) and which shows a negative result; one supporting *in Vitro* Mammalian Cell Gene Mutation Test (OECD TG 476) with the analogue substance (Distillates (petroleum), hydrodesulfurized middle (EC number 265-183-3, CAS RN 64742-80-9) and which shows an ambiguous result;
iv. One key *in vivo* cytogenicity/ bone marrow chromosome aberration (OECD TG 475) with an analogue substance (Distillates (petroleum), hydrodesulfurized middle (EC number 265-183-3, CAS RN 64742-80-9) and which shows a negative result;
v. A testing proposal for a Combined OECD TG 422/OECD TG 474 study.

However, you conclude that *in vitro* studies are not appropriate to address the mutagenicity properties of the Substance due to the difficulties with its solubility, and that *in vivo* studies are necessary to confirm the intrinsic properties of the Substance.

The *in vivo* study (see iv. above), provided on an analogue substance, does not provide compliant information on cytogenicity: there is, e.g. no information on (a) the mitotic index determined in at least 1000 cells/ all treated animal (including positive controls), untreated or vehicle/solvent negative control animal; on (b) at least 200 metaphases analysed for each animal for structural chromosomal aberrations including and excluding gaps.

For these reasons, ECHA agrees that further information is necessary for producing the chemical safety report and that this information can only be obtained by performing an *in vivo* mutagenicity study in accordance with Annex IX to REACH (Section 0.5, Annex I to REACH), to address gene mutation and chromosomal aberration.

Standard and modified Ames tests

You have submitted a sequential testing programme proposing to conduct new standard and modified Ames tests with the Substance. While ECHA acknowledges your intentions, it notes that a test covering an endpoint of Annex VII as you proposed does not fall within the scope of the examination of a testing proposal and that it is at your discretion to conduct such tests.

Combined OECD TG 422/OECD TG 474 study

As part of your "[redacted]" (November 2018) attached in the IUCLID dossier, section 7.5.3, you have submitted a proposal to test the Substance in a combined OECD TG 422/OECD TG 474 (*in vivo* micronucleus) study, oral route "*if any positive* in vitro *results*". You explained that *"due to the difficulties with solubility, it was considered that this endpoint [*in vitro *genotoxicity] was best addressed using an* in vivo *test. Further this would be incorporated into the planned OECD TG 422 test so as to minimise the use of animals. If the OECD TG 474 does not meet the full criteria for acceptance when conducted as part of other studies […], then a stand-alone study will be required."*

Your proposal for such a combined test cannot be accepted because: testing proposals can be only made for the provision of the information specified in Annexes IX and X to REACH. A test covering an endpoint of Annex VIII (combined repeated dose toxicity study with reproductive toxicity screening test (OECD TG 422)), as you proposed, does not fall within the scope of the examination of a testing proposal under Articles 40 and 10(a)(ix) of REACH.

Therefore, this part of the proposal is out of scope of the evaluation of your testing proposal. It is at your discretion to conduct such combined tests without compromising the validity of the test requested. In this regard, you must pay attention to the dosing schemes of the different studies, which may jeopardise the regulatory validity of each separate information requirement. Nonetheless, ECHA considers that you have adequately demonstrated the need to perform further testing to better address the lack of data for the endpoint of mutagenicity *in vitro/ in vivo*.

Test selection

According to ECHA Guidance Chapter R.7a, section R.7.7.6.3, information on the capability to induce gene mutations, structural chromosome aberrations (clastogenicity) and numerical chromosome aberrations (aneugenicity) is required to be able to evaluate the mutagenic potential of a substance.

The proposed *in vivo* micronucleus test (according to TG OECD 474) while it investigates *in vivo* chromosomal mutagenicity (as the study detects both structural and numerical chromosomal aberrations) is not suitable to address gene mutation.

According to the ECHA Guidance, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a genotoxicity indicator test that is suitable to address both gene mutation and chromosomal aberration, also for substances of low systemic bioavailability. However, the comet assay is not appropriate for detecting aneugens (numerical chromosome aberrations).

As indicated above, the *in vivo* mammalian erythrocyte micronucleus test ("MN" test, OECD TG 474) is a mutagenicity test that provides evidence on *in vivo* chromosomal mutagenicity, as the study detects both structural and numerical chromosomal aberrations.

Consequently, as also indicated in the ECHA Guidance, it is possible to combine the comet assay and the MN test into a single study. The combined study can help reduce the number

of tests performed and the number of animals used while addressing both structural and numerical chromosomal aberrations and gene mutations.

Therefore, the comet assay combined with the MN test is an appropriate study for the Substance.

Test design

According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combined test (OECD TG 489 and OECD TG 474) 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 line with the test method OECD TG 489, the test must 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)). Because of these expected or possible variables, you must analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

The combination of OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al*. 2011[2]).

Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or chromosome aberrations 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 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, under 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.

Outcome

According to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the proposed test under modified conditions, as explained above, with the Substance.

[2] Bowen D.E. *et al.* (2011). Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Mutation Research*, 722 7–19

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

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 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 the careful identification and description of the characteristics of the Tests Materials in accordance with OECD GLP (ENV/MC/CHEM(98)16) and EU Test Methods Regulation (EU) 440/2008 (Note, Annex), namely all the constituents must be identified as far as possible as well as their concentration. Also any constituents that have harmonised classification and labelling according to the CLP Regulation must be identified and quantified using the appropriate analytical methods.
   - Considering the toxicity of PAHs, you must report the total percentage and, as far as technically feasible, the individual percentages and identification of each PAH in the test material to assess whether it is representative of the Substance.

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[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 8 February 2019, following the necessary clarification of the identity of your substance.

ECHA held a third party consultation for the testing proposal from 14 May 2020 until 29 June 2020. ECHA did not receive information from third parties.

This testing proposal examination decision does not prevent ECHA from initiating compliance checks at a later stage on the registrations present.

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

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

In your comments on the draft decision, you made several remarks not relating to the Substance and the study requested in the present decision, but concerning other substances of the OtherGO category. More specifically:

- you indicated your understanding "*that no testing will be requested on the remaining OtherGO substances prior to the full clarification of the category*";
- you referred to comments relating to draft decision on another substance of the OtherGO category (i.e. concerning the route of administration for a prenatal developmental toxicity study; the selection of the test substance; and the test design of a extended one generation reproductive toxicity study)

However, these remarks are not relevant for the present decision. The present decision addresses exclusively the testing proposal you submitted specifically on the Substance. The purpose of the present decision is therefore not to assess your grouping strategy or real information needs for other substances of the OtherGO category. This will be assessed after the submission of the information requested in this decision and in decisions on other substances of the category. This assessment will also take account of other data that you may generate in order to support lower tier information on all the substances of the category.

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments and referred the modified draft decision to the Member State Committee.

You informed ECHA that you had no comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-74 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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)[6]**Error! Bookmark not defined.**

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.

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

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.

[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] 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) Data requirements to be fulfilled
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
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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.