EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 18 January 2021

Addressees
Registrants of TMAC CAS 1204-28-0 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
12/12/2019

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 4-chloroformylphthalic anhydride
EC number: 214-874-8
CAS number: 1204-28-0

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), ECHA requests that you submit the information listed below by the deadline of ***24 October 2022***.

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

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

1. *In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD TG 489) as requested below, with the option to combine it with the *in vivo* mammalian erythrocyte micronucleus test (test method: OECD 474), requested below.

B. Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method EU B.12./OECD 474); in rats, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

Conditions to comply with the requests

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 Annex VII of REACH, if you have registered a substance at 1-10 tonnes per annum (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa.

Registrants are only required to share the costs of information they are required to submit to

fulfil the information requirements for their registration.

ECHA requests the same Comet Assay from registrants at Annexes VII and VIII with the additional requirement to combine that test with another one at Annex VIII, while this combination is an option, not a requirement, at Annex VII. Only one study is to be conducted, taking into account the above requirements and option. 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.

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

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the 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.

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 requirements applicable to all the Registrants subject to Annex VII of REACH

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

1. ***In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2) with the option to combine it with an *in vivo* mammalian erythrocyte micronucleus test**

Under Annex VII Section 8.4., column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, *in vitro* cytogenicity test and *in vitro* gene mutation study in mammalian cells, which raise the concerns for gene mutations and chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

Therefore, you submitted a testing proposal for an *in vivo* mammalian alkaline comet assay combined with *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*. ECHA notes that 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 *in vivo* follow up genotoxicity study is necessary to address the concerns identified *in vitro*.

In your comments to the draft decision, you agree to this request.

i. Test selection

ECHA notes that the proposed test is appropriate to investigate effects on gene mutations and chromosomal aberrations *in vivo*[2].

The positive *in vitro* gene mutation study in bacteria and *in vitro* gene mutation study in mammalian cells available in the dossier indicate a concern for gene mutation. In the dossier there are also positive results for the *in vitro* cytogenicity test that indicate a concern for chromosomal aberration.

According to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the comet assay (OECD TG 489) that you propose is suitable to follow up the concern for gene mutation raised by the positive *in vitro* gene mutation study in bacteria (Annex VII, Section 8.4., column 2). Therefore, the comet assay is an appropriate follow-up test for the Substance.

In your testing proposal you also indicated the intention to combine the comet assay with the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474). ECHA considers that an *in vivo* micronucleus test is appropriate to investigate chromosomal aberrations (micronuclei) *in vivo* as described in the ECHA Guidance Chapter R.7a, Section R.7.7.1. and figure R.7.7-1. No *in vitro* cytogenicity study is required at Annex VII of REACH and the

[2] ECHA Guidance R.7a, Section R.7.7.6.3. and Figure R.7.7-1

additional *in vivo* micronucleus investigation you propose is not triggered under Annex VII. Therefore, it is at your discretion to combine the comet assay and the MN test into a single study. The combination of a comet assay with the MN test can help reduce the number of tests performed and the number of animals used while addressing both chromosomal aberration and gene mutation.

ECHA draws your attention to the fact that investigation *in vivo* of both gene mutations and chromosomal aberrations is triggered under Annex VIII Section 8.4., column 2 of REACH and that the comet assay combined with the MN test with the Substance is being requested from the Annex VIII registrant of this joint submission (see Appendix B.1).

ii. Test design

You proposed testing in rodents but did not specify the species to be used for testing. You proposed testing by the oral route.

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 line with the test method OECD TG 489, the test must be performed by analysing tissues from the liver as primary site of xenobiotic metabolism, and of 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.

You also proposed to analyse the kidney and bone marrow in the same study and to include a toxicokinetic investigation to confirm absorption of the Substance and tissue exposure. Performing these additional examinations is at your discretion. According to OECD TG 489, it may be useful to examine multiple tissues in the same animals provided that tissue selection is justified and the laboratory has demonstrated proficiency with those tissues and competency in handling multiple tissues at the same time. Furthermore, direct or indirect evidence supportive of exposure of the target tissue(s) are useful in the evaluation and interpretation of the study results.

In case you decide to perform the MN test in combination with the comet assay, you 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 the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al.* 2011[3]).

iii. Germ cells

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 in the comet assay, as it would optimise the use of animals. You can prepare the slides for male

[3] 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
[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

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.

iv. Outcome

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

Appendix B: Reasons for the requirements applicable to all the Registrants subject to 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 (Annex VIII, Section 8.4., column 2)

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.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, *in vitro* cytogenicity test and *in vitro* gene mutation study in mammalian cells, which raise the concerns for gene mutations and chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

Therefore, you submitted a testing proposal for an *in vivo* mammalian alkaline comet assay combined with *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*. ECHA notes that 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 *in vivo* follow up genotoxicity study is necessary to address the concerns identified *in vitro.*

In your comments to the draft decision, you agree to this request.

i. Test selection

ECHA notes that the proposed test is appropriate to investigate effects on gene mutations and chromosomal aberrations *in vivo*[5].

The positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

According to the ECHA Guidance R.7a, Section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a genotoxicity indicator test that is suitable to follow up the positive *in vitro* result for both chromosomal aberration and gene mutation. Moreover, 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 this study detects both structural and numerical chromosomal aberrations.

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 chromosomal aberration and gene mutation.

[5] ECHA Guidance R.7a, Section R.7.7.6.3. and Figure R.7.7-1

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

ii. Test design

You proposed testing in rodents but did not specify the species to be used for testing. You proposed testing by the oral route.

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 the liver as primary site of xenobiotic metabolism, and of 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.

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 the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al.* 2011[6]).

You also proposed to analyse the kidney and bone marrow in the same study and to include a toxicokinetic investigation to confirm absorption of the Substance and tissue exposure. Performing these intended additional examinations is at your discretion. According to OECD TG 489, it may be useful to examine multiple tissues in the same animals provided that tissue selection is justified and the laboratory has demonstrated proficiency with those tissues and competency in handling multiple tissues at the same time. Furthermore, direct or indirect evidence supportive of exposure of the target tissue(s) are useful in the evaluation and interpretation of the study results.

iii. Germ cells

You may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[7]) 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.

[6] 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
[7] 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

iv. Outcome

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

Appendix C: Procedural history

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 11 December 2019.

ECHA held a third party consultation for the testing proposals from 27 January 2020 until 12 March 2020. ECHA did not receive information from third parties.

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.

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.

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: Observations and technical guidance

1. This testing proposal examination decision does not prevent ECHA from initiating 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 your Member State(s).

3. Test guidelines, GLP requirements and reporting

   Under 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.

   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.

   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: 'How to report robust study summaries'[8].

4. Test material

   Selection of the test material(s)

   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"[9].

[8] https://echa.europa.eu/practical-guides
[9] https://echa.europa.eu/manuals

5. List of references of the ECHA Guidance and other guidance/ reference documents[10]

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)[11]

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

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

[10] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[11] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

Appendix E: List of the registrants to which the decision is addressed 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]

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.