EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 20 April 2021

Addressees
Registrants of JS_Methacrylicacid as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
19/09/2013

Registered substance subject to this decision ("the Substance")
Substance name: Methacrylic acid
EC number: 201-204-4
CAS number: 79-41-4

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***26 July 2022***.

Requested information must be generated using the Substance unless otherwise specified.

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

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: EU B.13/14. / OECD TG 471) using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102

2. If the test results in A.1 are positive, then: same in vivo genotoxicity study as also requested below in B.1 and C.1 (triggered by Annex VII, Section 8.4., column 2)

3. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201)

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

1. Same In vivo mammalian alkaline comet assay in rats, oral route, on the following tissues: liver, glandular stomach and duodenum as also requested below in C.1. (triggered by Annex VIII, Section 8.4., column 2)

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

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

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

Reasons for the request(s) are explained in the following appendices:

- Appendices entitled "Reasons to request information required under Annexes VII to X 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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and 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, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[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 VII of REACH

1. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is a standard information requirement in Annex VII to REACH.

You have provided a key study in your dossier:

i. Hasworth (1983) with the following strains, TA 98, TA 100, TA 1535 and TA 1537 which all gave negative results.

We have assessed this information and identified the following issue:

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471 (1997). One of the key parameters of this test guideline includes:

- The test must be performed with 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101)

The reported data for the study you have provided did not include results for the appropriate 5 strains, that is the required fifth strain, *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

The information provided does not cover one of the key parameters required by OECD TG 471.

Therefore, the information requirement is not fulfilled.

In your comments on the draft decision you agreed to perform the study.

Study design

To fulfil the information requirement for the Substance, the *in vitro* gene mutation study in bacteria (OECD TG 471) should be performed using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

2. In vivo mammalian alkaline comet assay

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.

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.*".

ECHA requests an *in vitro* test under Annex VII Section 8.4.1 (see Section A.1), which could raise a concern for gene mutation in case of positive results. In such a case, ECHA considers

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

that an appropriate *in vivo* follow up mutagenicity study would be necessary to address the potential gene mutation concern identified *in vitro*.

Therefore, if the test results in A.1 are positive, the same *in vivo* genotoxicity study requested in sections B.1 and C.1 is triggered. The selection of the requested test and the test design are addressed in request C.1.

3. Growth inhibition study aquatic plants

Growth inhibition study aquatic plants is an information requirement under Annex VII to REACH (Section 9.1.2.).

You have provided the following information:

i. OECD TG 201, key study, [redacted], 1999a;
ii. OECD TG 201, other: preliminary test with pH adjustment at 6.3, [redacted], 1999b;
iii. OECD TG 201, other: preliminary test with no pH adjustment, [redacted], 1999c;
iv. OECD TG 201, other: preliminary test with Ca^{2+} addition, [redacted], 1999d;
v. OECD TG 201, other: preliminary test with pH adjustment at 7, [redacted], 1999e;
vi. ISO 10253, supporting study, Sverdrup *et al.*, 2001;
vii. ISO 8692, other: screening test, Radix *et al.*, 2000;
viii. No guideline followed, other: short-term inhibition of *in vivo* fluorescence (direct fluorescence measurement), Radix *et al.*, 2000;
ix. No guideline followed, other: short-term inhibition of *in vivo* fluorescence (fuorescein diacetate (FDA) method), Radix *et al.*, 2000;
x. OECD TG 201, other: disregarded due to methodological deficiencies, [redacted], 1990.

With regard to the studies viii. and ix., we understand that you actually refer to an adaptation under Annex XI, Section 1.1.2. (Use of existing data not carried out according to GLP or the test methods referred to in Article 13(3) or REACH).

We have assessed this information and identified the following issues:

A. To fulfil the information requirement, a study must comply with OECD TG 201 and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test (Article 13(3) of REACH). Therefore, the following requirements must be met:

Validity criteria

- exponential growth in the control cultures is observed over the entire duration of the test;
- at least 16-fold increase in biomass is observed in the control cultures by the end of the test;
- the mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures is ≤ 35%;
- the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures is ≤ 7% in tests with *Pseudokirchneriella subcapitata* or *Desmodesmus subspicatus*. For other less frequently tested species, the value is ≤ 10%;

Technical specifications impacting the sensitivity/reliability of the test

- three replicates at each test concentration and at least three replicates for controls (including solvent controls, if applicable) are included;

- for *Pseudokirchneriella subcapitata,* the initial cell density is 5 x 10^3-10^4 cells/mL cells/mL;

Characterisation of exposure

- the concentrations of the test material are measured at least at the beginning and end of the test:
  1) at the highest, and
  2) at the lowest test concentration, and
  3) at a concentration around the expected EC_{50}.

  For volatile, unstable or strongly adsorbing test substances, additional samplings for analysis at 24 hour intervals are required.
- the test media prepared specifically for analysis of exposure concentrations during the test is treated identically to those used for testing (*i.e.* inoculated with algae and incubated under identical conditions);
- Algal biomass is determined based on dry weight per volume, or alternatively as cell counts or biovolume using microscopy or an electric particle counter. If an alternative method is used (*e.g.* flow cytometry, *in vitro* or *in vivo* fluorescence, or optical density), a satisfactory correlation with biomass must be demonstrated over the range of biomass occurring in the test.

Reporting of the methodology and results

- the results of algal biomass determined in each flask must at least daily during the test period are reported in a tabular form;

On study i. above:

- you report the following on the analytical monitoring of exposure concentrations: "*At the start of the test, samples were taken of each test solution, using the excess remaining after filling the test vessels*" and "*At the end of the test each blank solution was sampled and analysed*";
- on the test procedure, you have not specified: the composition of the test medium
- on the test design, you have not specified: the number of replicates culture at each test concentration and in the control(s)
- tabulated data on the algal biomass determined every 24 hours for each treatment group and control are not reported.

You specify that studies ii. to v. are preliminary tests to the study i. You provided only limited information on how these tests were conducted and you have not reported any information on the test results except the estimated effect values. You specify that these effects values were expressed as nominal concentrations while you report that losses ranging from 0 to 67% were measured in study iii. and v. and from 0 to 100% in study iv.

On study vi. above:

- the original publication specifies that a test according to ISO 10253 was conducted on *Skeletonema costatum*. In addition, non-guideline screening tests were conducted on *S. costatum* and nine additional marine algae species;
- in the study according to ISO 10253, the algal medium was analysed without an initial filtration and diethyl ether extraction was used. Exposure concentrations were not verified analytically in any of the additional screening tests;
- biomass was measured using *in vivo* fluorescence. No information on the relationship between measured *in vivo* fluorescence and biomass is provided;

- no information on algal cell density at the start of the test is reported;
- tabulated data, on the algal biomass determined every 24 hours for each treatment group and control, is not reported.

On study vii. above:

- the original publication specifies that these tests were conducted at an initial cell density of 5 x 10^5 cells/mL;
- no analytical verification of exposure concentration is reported and results were expressed as nominal concentrations;
- tabulated data, on the algal biomass determined every 24 hours for each treatment group and control, is not reported.

On study x. above:

- you consider the study unreliable as "*the study was performed in test medium without the carbonate/bicarbonate buffer. Therefore the results were achieved at low pH under non-guideline conditions*";
- you have not reported any information on the test design, the test procedure or the test results except the effects values;
- you state that "*After 96 h no detectable quantities of methacrylic acid were found in the test vessels. This might be due to volatility of the compound, adsorption to the glass and/or adsorption to particulate, including the algal cells in the test solution*".

Study i. does not include an appropriate analytical monitoring of exposure concentration as the test media used for the analysis of exposure concentrations was not inoculated with algae. As the test material is ionised at relevant pH and losses from the medium due to adsorption may be expected. Furthermore, you have only measured exposure concentrations at the start and end of the test while additional samplings for analysis at 24 hour intervals are required. Then, you have not provided adequate information to verify that the test design and test procedure comply with OECD TG 201. Finally, as you have not reported tabulated data on measured algal biomass, it is not possible to verify if the validity criteria of OECD TG 201 were met.

In your comments on the draft decision, you have attached an updated robust study summary for study i. which includes, among others, additional information on the analytical monitoring of exposure concentrations and tabulated data on the algal biomass determined every 24 hours for each treatment group and control. ECHA has assessed this new information against the requirement in OECD TG 201 and concludes that it is adequate to resolve the deficiencies identified above. The information you have provided in your comments therefore addresses the incompliances identified in this draft decision for this information requirement. However, until this information becomes available in your registration dossier, the information gap remains.

Then, the information provided on studies ii. and v. is insufficient to conduct an independent assessment of their relevance and of the reliability of the reported results. Furthermore, you have not demonstrated that the results of these studies can be reliably expressed based on nominal concentrations as your report that significant losses from the test medium were observed. As these studies are preliminary tests for study i., it is also likely that the deficiencies identified above also apply.

On study vi., the main test conducted on *S. costatum* did not include an appropriate monitoring of exposure concentration. Concentrations in metacrylic acid were obtained without removing the algae and following a solvent extraction. Therefore, this study

does not provide information on the concentration of the test material in true solution and the reported effect values may underestimate the toxicity of the test material. Then, no justification is provided that *in vivo* fluorescence was adequate for determination of biomass (*e.g.* evidence of correlation between the measured parameter and dry weight for both control and treated groups). The physiological status of algal cells is known to impact the efficiency of the non-photochemical quenching (NPQ) of fluorescence and differences in physiological status between treatments may bias the relationship between re-emitted fluorescence and biomass. Finally, without reporting tabulated data on measured algal biomass, it is not possible to verify if validity criteria consistent with the specifications of OECD TG 201 were met.

Study vii. was conducted at an initial biomass density 50-100 times higher than recommended by OECD TG 201 and may therefore have lower sensitivity. Furthermore, no measurement of exposure concentrations are reported. Finally, without reporting tabulated data on measured algal biomass, it is not possible to verify if validity criteria consistent with the specifications of OECD TG 201 were met.

The information provided on study x. does not allow an independent assessment of its relevance and of the reliability of the reported results.

B. Under Annex XI, Section 1.1.2., the information requirement may be adapted using existing data not carried out according to GLP or the test methods referred to in Article 13(3) or REACH if the following condition is met (among others):
   A. The study provides adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case OECD TG 201, including the concentrations of the test material leading to a 50 % and 0% (or 10%) inhibition of growth at the end of the test are estimated.

Studies viii. and ix. provide information on (i) relative *in vivo* fluorescence emission compared to control conditions and (ii) esterase activity of the cells and cell membrane integrity, respectively.

Therefore, these studies do not provide information on the key parameter foreseen to be investigated in an OECD TG 201 and your adaptation is rejected.

On this basis, none of the reported studies currently meet the information requirement. As already mentioned above, once the information provided in your comments is available in your registration dossier the information requirement will be regarded as fulfilled. However, until this information becomes available in your registration dossier, the information gap remains. You should therefore submit this information in an updated registration dossier by the deadline set in the decision.

Study design

The Substance is difficult to test as it is ionisable and therefore may have high adsorption potential. OECD TG 201 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. If it is not possible to demonstrate the stability of exposure concentrations (i.e. measured concentration(s) not within 80-120%

of the nominal concentration(s)), you must express the effect concentration based on measured values as described in OECD TG 201. In case a dose-response relationship cannot be established (no observed effects), you must demonstrate that the approach used to prepare test solutions was adequate to maximise the concentration of the Substance in the test solution.

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

1. In vivo mammalian alkaline comet assay

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.

The ECHA guidance R.7a 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 a positive result for the *in vitro* cytogenicity test which raises the concern for chromosomal aberrations.

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

The assessment of the information provided to fulfil this information requirement, the selection of the requested test and the test design are addressed in request C.1.

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

1. In vivo mammalian alkaline comet assay

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.

In relation to the first condition, your dossier contains positive results for the *in vitro* cytogenicity test in mammalian cells (Schweikl, 2001) which raises the concern for chromosomal aberrations.

In relation to the second condition, you have provided data from the following sources of information as part of an adaptation under section 1.2 of Annex XI (weight of evidence):

i. [redacted] (1986), similar to OECD TG 478 (Rodent Dominant Lethal Test) with the analogue methyl methacrylate.
ii. [redacted] (1976), similar to OECD TG 475 (Mammalian Bone Marrow Chromosome Aberration Test) with the analogue methyl methacrylate.
iii. [redacted] (1979), similar to OECD TG 475 (Mammalian Bone Marrow Chromosome Aberration Test) with the analogue methyl methacrylate.
iv. Hachiya (1982), similar to OECD TG 474 (Mammalian Erythrocyte Micronucleus Test) with the analogue methyl methacrylate.

Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the (dangerous) property investigated by the required study.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach.

However, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has or has not a particular dangerous property.

In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation and identified the following issues.

To fulfil the information requirement, normally a study performed according to OECD TG 474, 475 or 489, must be provided[3]. OECD TGs 474/475 investigate structural and numerical chromosomal aberrations while OECD TG 489 recognises primary DNA damage that would

[3] ECHA Guidance R.7a, R.7.7.6.3, p. 568

lead to gene mutations and/or chromosomal aberrations. These *in vivo* test methods provide information on somatic cells in animals.

Source of information (i.) does not investigate chromosomal aberrations in somatic cells but in germ cells. Therefore, this study does not provide information that would contribute to the conclusion on the information investigated by the required studies.

The sources of information (ii.) to (iv.), provide relevant information on chromosomal aberrations in somatic cells of animals.

However, the reliability of these sources of information is significantly affected by the following deficiencies:

A. The specifications/conditions of OECD TG 474 or 475 include: (a) the collection of bone marrow at two different times after single treatment; (b) the scoring of at least 4000 immature erythrocytes per animal (OECD TG 474) / the analysis of at least 200 metaphases for each animal (OECD TG 475).

   However, the reported data for studies (ii.) to (iv.) indicates that (a) the bone marrow was collected only 24 hours after single treatment; (b) only 2000 erythrocytes were evaluated per animal (OECD TG 474) / only 50 cells per animal were analysed (OECD TG 475).

   As indicated in OECD TGs 474/475, this information is required to establish the acceptability of the test. As explained above, the appropriate number of cells was not analysed, hence the acceptability criteria are not fulfilled. Therefore the test results, provided in studies ii. to iv., cannot be considered as reliable sources of information that could contribute to the conclusion on chromosomal aberrations in somatic cells investigated by the required studies.

B. Pursuant to Article 10(a)(vii) of the REACH Regulation, the information set out in Annex VII to XI must be provided in the form of a robust study summary. Article 3(28) defines a robust study summary as a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report.

   However, for the source of information (iv.) you have not provided adequate and reliable documentation in a form of a robust study summary, as required by Article 10(a)(vii) and Article 3(28).

Taken together, even if the sources of information (ii.) to (iv.) provide information on chromosomal aberrations in somatic cells in animals, their reliability is affected so significantly that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in OECD TGs 474/475/489. Therefore, your adaptation is rejected and the information requirement is not fulfilled.

Therefore, the conditions set out in Annex IX, Section 8.4, column 2 are met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

In your comments on the draft decision, you acknowledged that the dossier of your Substance contains positive results for the *in vitro* cytogenicity tests and the *in vitro* gene mutation studies in mammalian cells with the analogue substance methyl methacrylate (MMA). However, you argue that almost all of the weak positive results in these *in vitro* studies were reached at cytotoxic concentrations leading to your assessment that MMA "is a high toxicity clastogen (i.e. induction of chromosomal aberrations is bound to highly toxic doses)". In addition, you state that the observations were done in pre-guideline studies with various deviations when compared against current guidelines. Therefore, you intend to perform a new set of relevant *in vitro* guideline compliant studies with your Substance before a decision is made on further animal testing.

In addition, in your comments to the proposal for amendments (PfAs) submitted by one of the Member States Competent Authorities (MSCAs) you indicate that you have initiated the *in vitro* battery tests with the Substance. Based on the first draft reports, concerning OECD TG 471 and OECD TG 476 studies, it seems that there is no concern for gene mutation and you also expect a negative outcome for the OECD TG 487.

We acknowledge that you intend to fulfil the information on Mutagenicity required by REACH with additional *in vitro* mutagenicity studies. We also note that you refer to negative *in vitro* results; however this testing programme is incomplete. You have not provided in your comments any scientific data that could be evaluated by ECHA to confirm these results. You may, under your own responsibility, continue to carry out your testing programme. If it fails and the resulting data yields positive results you remain responsible for providing the *in vivo* information requested by this decision by the set deadline. ECHA will evaluate all the later information in the follow up evaluation after passing of the date set out on the first page of this decision.

On the basis of the scientific data included in your registration dossier and those included in your comments ECHA maintains its conclusion that the conditions set out in Annex IX, Section 8.4, column 2 are met and therefore information on an appropriate *in vivo* somatic cell genotoxicity study is required.

The information provided in the assessed technical dossier, and in your comments on either the draft decision or the PfAs, are further not sufficient to fulfil the information requirement.

I. Test selection

As indicated above, according to the ECHA Guidance Chapter R.7a[4], the mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) or the mammalian bone marrow chromosomal aberration test ("CA 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 ("comet assay", OECD TG 489) is a suitable test to be performed.

However, in view of the reactivity of the Substance, there is a concern for mutagenicity at the initial site of contact tissues, which cannot be evaluated by performing an OECD TG 474/475, since these studies only measure effects in the bone marrow (distant tissue). Moreover, the bone marrow would likely not be reached by the Substance due to its reactivity. Therefore, neither the MN test or CA test are suitable for the Substance. The comet assay, on the other hand, enables the generation of information regarding genotoxic effects at the site of contact.

[4] ECHA Guidance Chapter R.7a, Section R.7.7.6.3

The comet assay is further suitable to follow up positive *in vitro* results for gene mutations and chromosomal aberrations. Therefore, the *in vivo* comet assay is the suitable test for the Substance, to follow up the chromosomal aberration concern already identified and a potential gene mutation concern which could be raised by the test requested under section A.1 (see the conditional request under A.2).

II. Test design

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

In your comments to the PfA submitted by one of the MSCAs you indicate that the PfA would have led to a modification of the requested information from inhalation to oral route. ECHA clarifies that no modification occurred, as already the request in the initial draft decision, notified to you in accordance with Article 50(1), referred to oral route of administration. 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.

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)). 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.

III. 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 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, 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.

2. Long-term toxicity testing on aquatic invertebrates

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

You have provided the following information:

i. OECD TG 211, key study, [redacted], 1995;
ii. No guideline followed, other: "*Screening test (short-term chronic test)*", Radix *et al.*, 2000;

With regard to the studies ii., we understand that you actually refer to an adaptation under Annex XI, Section 1.1.2. (Use of existing data not carried out according to GLP or the test methods referred to in Article 13(3) or REACH).

We have assessed this information and identified the following issue:

A. To fulfil the information requirement, a study must comply with the OECD TG 211 and the requirements of OECD GD 23 (ENV/JM/MONO(2000)6/REV1) if the substance is difficult to test (Article 13(3) of REACH). Therefore, the following requirements must be met:

Validity criteria
- the mean number of living offspring produced per parent animal surviving is ≥ 60 at the end of the test;

Technical specifications impacting the sensitivity/reliability of the test
- the test medium fulfils the following condition: total organic carbon (TOC) ≤ 2 mg/L;

Reporting of the methodology and results
- the test procedure is reported (*e.g.* loading in number of *Daphnia* per litre, test medium composition);
- the full record of the daily production of living offspring during the test in each replicate is provided;

On study i. above:
- you have not reported information on time to first brood in the controls and at each test concentration;
- on the test procedure, you have not specified the composition of the test medium;
- the full record of the daily production of living offspring during the test in each replicate is not provided;
- the number of deaths among the parent animals (if any) and the day on which they occurred is not reported;

Therefore, without information on daily production of living offspring during the test in each replicate, it is not possible to verify study i. meets the validity criteria of OECD TG 211. Furthermore, information on time to first brood is not provided and therefore this study does not cover all key parameters of the test guideline. Finally, you have not provided information on the test medium composition and it cannot be verified if its TOC content comply with the test guideline requirements.

In your comments on the draft decision, you have attached an updated robust study summary for study i. which includes, among others, additional information on the TOC content of the dilution water and adequate reporting on time to first brood, the daily production of living offspring and number of deaths among the parent animals the controls and at each test concentration. ECHA has assessed this new information against the requirements in OECD TG 211. Despite the study having some deficiencies (reproductive output in the control slightly below the minimum requirement, selection of dose not optimal), ECHA has concluded that this study allows identifying a NOEC for Daphnia reproduction efficiency and that the provided information is adequate to resolve the deficiencies identified above. However, until this information becomes available in your registration dossier, the information gap remains.

B. Under Annex XI, Section 1.1.2., the information requirement may be adapted using existing data not carried out according to GLP or the test methods referred to in Article 13(3) or REACH if the following condition is met (among others):
   - The study provides adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case OECD TG 211, including the concentrations of the test material leading to no observed effect (NOECs) on the following parameters are estimated:
     1) the reproductive output of *Daphnia* sp. expressed as the total number of living offspring produced at the end of the test, and
     2) the survival of the parent animals during the test, and
     3) the time to production of the first brood.

   Study ii. corresponds to a non-guideline test investigating *Brachionus calyciflorus* reproductive output following a two days exposure period.

   Hence, this study does not provide adequate and reliable coverage of the key parameters of OECD TG 211 as it was not conducted on Daphnia sp. Furthermore, it cannot be regarded as a long-term test due to the short exposure period. Therefore, your adaptation is rejected.

On this basis, none of the reported studies currently meet the information requirement. As already mentioned above once the information provided in your comments is available in your registration dossier the information requirement will be regarded as fulfilled. However, until this information becomes available in your registration dossier, the information gap remains. You should therefore submit this information in an updated registration dossier by the deadline set in the decision.

Study design

OECD TG 211 specifies that for difficult to test substances OECD GD 23 must be followed. As already explained above, the Substance is difficult to test. Therefore, you must fulfil the requirements described in 'Study design' under Section A.2.

Appendix D: 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[5].

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 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[6].

[5] https://echa.europa.eu/practical-guides
[6] https://echa.europa.eu/manuals

Appendix E: Procedure

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

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

The compliance check was initiated on 7 August 2019.

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

ECHA took into account your comments and did not amend the request(s).

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 amendment(s) and referred the modified draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-73 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix F: List of references - ECHA Guidance[7] 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)[8]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[8]

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.

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

OECD Guidance documents[9]
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.

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

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

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

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

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