EUROPEAN CHEMICALS AGENCY

Helsinki, 18 December 2017

Addressee: [redacted]

Decision number: CCH-D-2114382275-45-01/F
Substance name: acrylic acid, monoester with propane-1,2-diol
EC number: 247-118-0
CAS number: 25584-83-2
Registration number: [redacted]
Submission number: [redacted]
Submission date: 1.2.2016
Tonnage band: [redacted]

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA requests you to submit information on:

1. **Name(s) in the IUPAC nomenclature or other international chemical name(s) (Annex VI, Section 2.1.1.) of the registered substance;**
2. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26./OECD TG 408) in rats with the registered substance;**
3. **In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, by gavage, on the following tissues: liver, glandular stomach and duodenum with the registered substance; The test material used should be freshly prepared.**

   OR

   Transgenic rodent somatic and germ cell gene mutation assays (Annex X, Section 8.4., column 2; test method: EU B.58./OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver, glandular stomach with the registered substance; germ cells and duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative. The test material used should be freshly prepared.
4. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route; with the registered substance;**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**
5. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species, rabbits, oral route with the registered substance.**

You are required to submit the requested information in an updated registration dossier by **25 March 2021** except for the information requested under point 2 for a sub-chronic toxicity study (90-day) which shall be submitted in an updated registration dossier by **3 January 2019**. You may only commence the extended one-generation reproductive toxicity study as requested under point 4 after **25 March 2019**, unless an indication to the contrary is communicated to you by ECHA before that date. You shall also update the chemical safety report, where relevant. The timelines have been set to allow for sequential testing.

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

Applicable only for the final decision: 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, shall 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.

Authorised[1] by Claudio Carlon, Head of Unit, Evaluation E2.

[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 1: Reasons

0. Grouping of substances and read-across approach

Article 13(1) of the REACH Regulation provides that information on intrinsic properties of substances may be generated by means other than tests. Such other means include the use of information from structurally related substances (grouping of substances and read-across), "provided that the conditions set out in Annex XI are met".

In the registration, you sought to adapt the standard information requirements for

- Genetic toxicity (Annex X, Section 8.4)
- Repeated dose toxicity (Annex IX, Section 8.6.2.)
- Reproductive toxicity (Annex X, Section 8.7.)
- Prenatal developmental toxicity (Annex X, Section 8.7.2.)
- Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

by applying a read-across adaptation following REACH Annex XI, Section 1.5.

The source substances are 2-hydroxyethyl acrylate (HEA) CAS RN 818-61-1 (repeated dose toxicity), methyl acrylate CAS RN 96-33-3 and acrylic acid CAS RN 79-10-7 (prenatal developmental toxicity and reproductive toxicity).

Annex XI, Section 1.5. requires a structural similarity among the substances within a group or category and that relevant properties of a substance within the group can be predicted from the data on reference substance(s) within the group by interpolation. The following analysis presents your justification for the proposed grouping approach and read-across hypothesis, together with ECHA's analysis concerning the justification in both a generic and a property-specific context.

0.1 Description of the grouping and read-across approach proposed by the Registrant

You have provided a read-across justification as a separate attachment in the updated registration in section 13 of the IUCLID dossier. In the justfication you summarised the arguments to support the read-across approach as following:

"*The target chemical hydroxypropyl acrylate (CAS no. 25584-83-2) and its analogue chemicals 2-hydroxyethyl acrylate (CAS no. 818-61-1), acrylic acid (CAS no. 79-10-7), and methyl acrylate (CAS no. 96-33-3) have a similar molecular structure, i.e. all substances are acrylates. In addition hydroxypropyl acrylate and 2-hydroxyethyl acrylate contain an alcohol group which only differs in the length of the alcohol being either an propyl or a ethyl. The functional groups of the compounds are the acrylate and, where applicable, the alcohol group. The difference in length of the backbone is toxicologically of lesser importance. In addition, metabolism of the target and source chemicals will be similar. Finally, the substances have similar physico-chemical and toxicological properties.*"

ECHA considers this as the hypothesis under which you make predictions for the properties listed above.

0.2 ECHA analysis of the grouping and read-across approach in light of the requirements of Annex XI, Section 1.5.

With regard to the proposed predictions ECHA has the following observations:

(i) Substance identification and characterisation of source and target substances

The substance identification and characterisation of the source and target need to be sufficiently detailed in order to assess whether the attempted prediction is not compromised by the composition and/or impurities. In the ECHA practical guide 6 "How to report on Read-Across" it is recommended to follow ECHA's "*Guidance for identification and naming of substances under REACH and CLP*" (version 2.1, May 2017). This ensures that the identity of the source and target substance and their impurity profile allows an assessment of the suitability of the substances for read-across purposes.

The source substances are 2-hydroxyethyl acrylate (HEA) CAS RN 818-61-1 (repeated dose toxicity), and methyl acrylate CAS RN 96-33-3 and acrylic acid CAS RN 79-10-7 (prenatal developmental toxicity and reproductive toxicity). The test substance purity is reported as 94% or higher.

The registered substance (Hydroxypropylacrylate or HPA) is composed of approx. [redacted] % [redacted] and approx. [redacted] % [redacted] with impurities of less than 1%. However, ECHA notes that the name and other identifiers reported for the registered substance are not consistent and the identification of the registered substance is ambiguous. In order to ensure that potential hazardous properties of the substance are not underestimated, the substance identification deficiencies must be resolved before identifying the test sample to be used for the testing requested in the present decision (see Appendix 1, section 1).

Therefore, the suitability of the read-across between target and source substances cannot be assessed.

(ii) Support of a similar or regular pattern as a result of structural similarity

In order to meet the provisions in Annex XI, Section 1.5. to predict human health effects from data for a reference substance within the group by interpolation to other substances in the group, ECHA considers that structural similarity alone is not sufficient. It has to be justified why such prediction is possible in view of the identified structural differences and the provided evidence has to support such explanation. In particular, the structural similarities must be linked to a scientific explanation of how and why a prediction is possible.

ECHA notes that the registered substance and the analogue substance display significant structural differences, especially with regard to acrylic acid and methyl methacrylate used to adapt the reproductive toxicity, prenatal developmental toxicity and the ethyl acrylate used in the genetic toxicity. ECHA concludes that with regard to prenatal developmental toxicity, reproductive toxicity and genetic toxicity, you have not addressed the obvious structural differences between the source substances and the target substance and did not explain why those differences would not lead to differences in the toxicity profile of target and source substances. In the absence of a justification that addresses these differences, the provided documentation cannot be considered to establish a valid and scientifically credible link between the structural similarity and the prediction.

The comparison between the HEA and HPA, which also have a closer structural similarity compared to the analogues used for the reproductive and genetic toxicity endpoints, seems plausible for the repeated dose toxicity via the inhalation route because inhalation studies (28d) are available for target and source substance and they show comparable effects (local irritation in the respiratory tract). However, ECHA considers that with regard to subchronic toxicity testing, the oral route is the most appropriate because the substance is a liquid of very low vapor pressure and no uses with spray application are reported that could potentially lead to aerosols of inhalable size. For the oral route the read-across cannot be supported because a comparison between the toxicities after oral administration between HEA and HPA was not possible as the dossier did not contain reliable oral studies for the source and there were no oral studies at all for the target substance. Comparison of the prenatal developmental toxicity and reproductive toxicity between the source and the target was not possible due to lack of relevant studies with the target substance.

(iii) Toxicokinetics

One important aspect in establishing that substances have similar effects or follow a regular pattern is the comparison of absorption, distribution, metabolism and elimination of source and target substances. This allows assessing the qualitative and quantitative internal systemic exposure of the test organism when exposed to source and target, respectively. ECHA observes that in your justification document you have provided a toxicokinetic assessment of the source and the target substances. However, ECHA considers that you have not addressed sufficiently the potential implications of the differences in the metabolites (ethylene glycol vs propylene glycol). You state: "*None of the harmonized classification and ECHA disseminated dossiers indicate that the metabolites are genotoxic, carcinogenic, or reprotoxic*".

The fact that there is no harmonised classification is not a guarantee that the substances behave similarly with regard to, e.g., subchronic toxicity.

In your comments to the draft decision, you have included references to several *in vitro* and *in vivo* studies on the toxicokinetics relating to lower acrylates, such as ethyl and methyl acrylates, and their rates of hydrolysis. Also, kinetics studies on hydroxyethyl acrylate are included. You also state that there is an ongoing plasma kinetic study in Wistar rats with oral administration to confirm the fast enzymatic hydrolysis of hydroxypropyl acrylate to acrylic acid and propylene glycol. ECHA notes that this information will be evaluated whenever it becomes available in form of a dossier update.

0.3 Conclusion on the read-across approach

The adaptation of the standard information requirements for the endpoints subchronic toxicity, genetic toxicity, prenatal developmental toxicity and reproductive toxicity in the technical dossier is based on the proposed read-across approach examined above. ECHA does not consider the read-across justification to be a reliable basis to predict the properties of the registered substance for the reasons set out above.

Pursuant to Article 41(1) of the REACH Regulation, ECHA concludes that the adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. Therefore, ECHA rejects all adaptations in the technical dossier that are based on Annex XI, Section 1.5.

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1. Name or other identifier of the substance (Annex VI, Section 2.1.)

Pursuant to Article 10(a)(ii) of the REACH Regulation, the technical dossier shall contain information on the identity of the substance as specified in Annex VI, Section 2 of the REACH Regulation. In accordance with Annex VI, Section 2 the information provided shall be sufficient to enable the identification of the registered substance.

According to the *Guidance for identification and naming of substances under REACH and CLP* (version 2.1, May 2017), thereinafter referred to as "the Guidance":

Multi-constituent substances are those where more than one well-defined constituent is present in a concentration ≥10% (w/w) and <80% (w/w).

A multi-constituent substance is named as a reaction mass of the main constituents of the substance as such i.e. not the starting materials needed to produce the substance.

The IUPAC name given to the registered substance ("[redacted]") indicates a well defined substance consisting of the two constituents "[redacted]" and "[redacted]" present in concentrations between [redacted] and [redacted] % w/w. This is in contrast to other parts of the dossier, notably the EC and CAS number and the reported composition, that describe the registered substance as containing the two main constituents "[redacted]" and "[redacted]".
ECHA therefore concludes that the name and other identifiers reported for the registered substance are not consistent and the identification of the registered substance is ambiguous.

You need to ensure that the name and other identifiers of the substance are used consistently throughout the dossier. Please review all chemical names and identifiers given in your dossier for consistency and correct them if necessary.

As for the reporting in the registration dossier, the information should be included as appropriate in all IUCLID sections, especially sections 1.1, 1.2, and 1.4.

ECHA Secretariat acknowledges the comments received regarding this issue and the corrected name given therein ("[redacted]"). The name proposed describes the registered substance in a consistent way.

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

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(e) and 13(4) of the REACH Regulation, a technical dossier registered at [redacted] shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

A "sub-chronic toxicity study (90 day)" is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

There is no sub-chronic toxicity study (90 days) conducted with the registered substance.

For repeated dose toxicity via inhalation you have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing the

following study records for a with the analogue substance 2-hydroxyethyl acrylate (CAS RN 818-61-1):

Inhalation exposure: Results of a two year inhalation toxicity study of HEA in rats (1979), effects of repeated inhalation exposures of rats and single exposure of humans to vapors of HEA vapors (1970).

Effects of repeated inhalation exposures of rats and single exposure of humans to vapors of HEA vapors.

Repeated (28 days) Inhalation Toxicity of HPA in rats, mouse, rabbits and dogs.

The studies are pre-GLP and non-guideline and are therefore considered of limited reliability. In any case, as explained further below, ECHA considers that, based on the physico-chemical properties of the substance as well as on the provided uses, the oral route is the most appropriate route of exposure, and ECHA therefore requires information on oral toxicity

For repeated oral toxicity, you have claimed an adaptation according to Annex XI, Sections 1.2. and 1.5. You have provided the following studies: Results of 100-day dietary feeding studies of 2-hydroxyethyl acrylate monomer and QX 3820 polymer latex in rats and beagle hounds (1967).

ECHA considers that the studies submitted for repeated dose toxicity, oral route, are not adequate in terms of study design. Both the rat and the dog studies were conducted before GLP (1967) and they do not conform to modern test guidelines. The animal number is only 10/ sex/ dose in the rat study and 2/ group in the dog study and you confirmed that the "*documentation [is] not up to today's standards*". The rationale for the dose level setting is unclear and the doses seem too low as there were no signs of toxicity even in the highest dose.
Furthermore, as explained in Section 0, since no repeated dose toxicity studies are available for HPA via oral route it is not possible to compare the toxicities of the target substance and source substance.

Therefore, your adaptation of the information requirement is rejected. ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a (version 6.0, July 2017), section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is a liquid of very low vapour pressure and no uses with spray application are reported that could potentially lead to aerosols of inhalable size.

Hence, the test shall be performed by the oral route using the test method EU B.26./OECD TG 408.

According to the test method EU B.26./OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

In your comments to the draft decision you express your disagreement on the most appropriate route of exposure. You have also included toxicological summaries of data on hydroxypropyl and hydroxyethyl acrylate, acrylic acid and propylene glycol. Some of the data was already in the original submission, whereas new data on acrylic acid, propylene glycol, butyl and methyl acrylate has been included.

ECHA notes that it maintains its view regarding the most relevant route of exposure. ECHA considers that even though exposure at work place predominantly occurs dermally or even via the inhalation route, the uncertainties of the substance reaching the body, e.g., due to poor absorption or low intake via the inhalation route, would lead to uncertainties in the hazard profile especially regarding the systemic effects. ECHA further notes that it will evaluate the new information referred to by you during the follow up after it becomes available in the form of a dossier update.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Repeated dose 90-day oral toxicity study (test method: EU B.26./ OECD TG 408) in rats.

3. In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2) or Transgenic rodent somatic and germ cell gene mutation assays (Annex X, Section 8.4., column 2)

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(e) and 13(4) of the REACH Regulation, a technical dossier registered at [redacted] shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex X, Section 8.4. provides that "If there is a positive result in any of the in vitro genotoxicity studies in Annexes VII or VIII, a second in vivo somatic cell test may be necessary, depending on the quality and relevance of all the available data."

The dossier contains two OECD TG 473 (*In vitro* Mammalian Chromosome Aberration Test) tests, which were positive with and without metabolic activation. The Registrant has performed an *in vivo* micronucleus test OECD TG 474 (Mammalian Erythrocyte Micronucleus Test) which gave a negative result. The ratio of polychromatic erythrocytes to normochromatic erythrocytes was slightly affected by the treatment with 2-hydroxypropyl acrylate at a dose of 600 mg/kg bw, which can be considered as evidence of the substance reaching the bone marrow. Therefore, the positive result in chromosomal aberrations *in vitro* can be considered adequately followed up.

However, because the test in *Escherichia coli* WP2/pKM101, WP2 uvrA/pKM101 (Watanabe *et al.*, 1996) with an assigned reliability score of 2 (not specified as being GLP compliant) was also positive in both *E. coli* strains, a second *in vivo* test may be indicated to follow up on gene mutation mutagenic mode of action.

You have added supportive evidence of lack of mutagenicity by providing the results of a chronic inhalation toxicity study with 12-month interim sacrifices for cytogenetic examinations including analysis of chromosomal aberrations with HEA, an analogue used as a read across source substance. However, this assay examines chromosome aberrations, and is not informative about the substance's potential to cause gene mutations. Therefore, it cannot be used to follow up the positive result of the Ames test.

ECHA notes that the study analysed a very limited number of metaphase cells when compared to the OECD TG 475 requirement (50 metaphases were analysed when at least 200 metaphases should be analysed for each animal for structural chromosomal aberrations

according to OECD TG 475). In addition, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

You also included a recent TGR assay conducted with ethyl acrylate but the study cannot be used to cover the information requirements for this endpoints as the apropriateness of the read-across could not be assessed (please see section 0). According to the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation.

Hence, ECHA considers that the TGR and the comet assay are suitable tests to follow up the concern on gene mutation for the substance subject to the decision. In case you decide to perform the TGR assay according to the test method EU B.58/OECD TG 488, the test shall be performed in transgenic mice or rats and the substance is usually administered orally. In case you decide to perform the comet assay according to the test method OECD TG 489, the test shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

According to the test method EU B.58/OECD TG 488, the test shall be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum as rapidly proliferating tissue and site of direct 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract. However, duodenum shall be analysed if the results of the glandular stomach and of the liver are negative.

Male germ cells shall be collected at the same time as the other tissues (liver and glandular stomach), and stored up to 5 years (at or below −70ºC).

This duration is sufficient to allow the Registrant or ECHA, in accordance to Annex X, Section 8.4., column 2, to decide on the need for assessment of mutation frequency in the collected germ cells.

According to the test method (OECD TG 489), the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism and 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

In your comments to the draft decision, you note that a new Ames test has been conducted under GLP conditions and according to the latest test guideline (OECD 471) with HPA. This new information is however not available in the dossier (submission number [redacted]).

Following the proposal for amendment made by one Member State Competent Authority, ECHA considers that it is useful to take into account the cross-linking properties of the registered substance in the experimental setup of the comet assay. Therefore, you may consider to prepare and analyse two sets of slides when performing the comet assay:

- one set submitted to the standard experimental conditions (as described in OECD TG 489);
- the other set of slides submitted to modified experimental conditions that enable the detection of DNA crosslinks; the modified experimental conditions may utilise one of the following options: (i) increase of electrophoresis time, e.g. as described in the reference 23 of the TG 489[2] or (ii) treatment of isolated cells (either in suspension or embedded in the slides) [to induce additional DNA damage] with a chemical (e.g. MMS) or (iii) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options ii and iii are described e.g. in the references 36-39 of the TG 489[3] or Pant *et al.*, 2015)[4].

The modified protocol to detect crosslinks shall include a positive control to ensure the robustness of the test result obtained on the registered substance: an additional group of animals shall be treated with a known cross-linking substance (e.g. hexamethyl phosphoramide or cisplatine).

In your comments to the Member States' proposals for amendment (PfAs) you considered that the Ames test with a positive result (Watanabe *et al.*, 1996) has a questionable reliability because the purity of the test material is not specified. ECHA notes that a reliability 2 was assigned to this study, which is consistent with the consideration about purity. Hence this study is adequate for assessment.

In your comments, you also expressed concern on the appropriateness of asking *"for non-validated test methods or non-validated deviations from a test guideline within a compliance check. Results of such tests might be regarded as questionable or non-reliable and trigger further animal testing."* However, ECHA does not consider the modified experimental conditions to be deviations from the Test Guideline. In fact, the OECD TG 489 (adopted 29 July 2016) in paragraph 11 provides references to studies using modified experimental conditions of the comet assay that detected crosslinks. In addition to the references given in the guideline, ECHA has added an additional reference (Pant *et al.*, 2015) for guidance on such modification to this decision. That is why ECHA recommends you to consider the modified experimental conditions, should you decide to conduct the study.

Moreover, in your comments, you have provided a summary of the study ([redacted], 2016). ECHA notes that the study is conducted according to OECD TG 471 and GLP. The study

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[2] (23) Nesslany, F, Zennouche N, Simar-Meintieres S, Talahari I, NKili-Mboui E-N, Marzin D (2007), *In vivo* Comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, Vol. 630/1, pp. 28-41.

[3] (36) Merk, O., G. Speit (1999), Detection of crosslinks with the Comet assay in relationship to genotoxicity and cytotoxicity, *Environmental and Molecular Mutagenesis*, Vol. 33/2, pp. 167-72;
(37) Pfuhler, S., H.U. Wolf (1996), Detection of DNA-crosslinking agents with the alkaline Comet assay, *Environmental and Molecular Mutagenesis*, Vol. 27/3, pp. 196-201;
(38) Wu, J.H., N.J. Jones (2012), Assessment of DNA interstrand crosslinks using the modified alkaline Comet assay, *Methods in Molecular Biology*, Vol. 817, pp. 165-81;
(39) Spanswick, V.J., J.M. Hartley, J.A. Hartley (2010), Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay, *Methods in Molecular Biology*, Vol. 613, pp. 267-282.

[4] Pant K, Roden N, Zhang C, Bruce C, Wood C, and Pendino K (2015) Modified In Vivo Comet Assay Detects the Genotoxic Potential of14-Hydroxycodeinone, an a,b-Unsaturated Ketone in Oxycodone. *Environmental and Molecular Mutagenesis* 56, 777-787.

examined the mutagenic potential of hydroxypropylacrylate (purity 99.6%) in the following strains: *S. typhimurium* TA 1535, TA 100, TA 1537, TA 98 and *E. coli* WP2 uvrA.
The study concluded that "*under the experimental conditions of this study, the test substance Hydroxypropylacrylate is not mutagenic in the* Salmonella typhimurium/Escherichia coli *reverse mutation assay in the absence and the presence of metabolic activation.*"

ECHA notes that the *E. coli* strain used in the new study (WP2 uvrA) is one of the strains recommended for a standard 5-strain Ames test. ECHA however observes this strain is not among the ones recommended by the guideline specifically for detection of cross-linking mutagens. According to the OECD TG 471 "*In order to detect cross-linking mutagens it may be preferable to include TA102 or to add a DNA repair-proficient strain of* E. coli [e.g. *E. coli* WP2 or *E.coli* WP2 (pKM101).]". ECHA furthermore notes that you did not include in your new Ames test any strain that were used in the study by Watanabe *et al.* (1996). ECHA considers that with the remaining uncertainty of the possible differences in sensitivities in the different strains of *E. coli*, it cannot disregard the positive result found by Watanabe *et al.* (1996).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision:

Transgenic rodent somatic and germ cell gene mutation assays (test method: EU B.58/ OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver *and* glandular stomach; germ cells and duodenum shall be harvested and stored for up to 5 years; duodenum shall be analysed if the results of the glandular stomach and of the liver are negative. The test material used should be freshly prepared.

or

In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, by gavage, on the following tissues: liver, glandular stomach and duodenum with the registered substance. The test material used should be freshly prepared.

Note for your consideration

You should consider performing an Ames study using the strains recommended to detect crosslinking mutagens, i.e., *E. coli* WP2 or *E. coli* WP2 (pKM101) or TA102 which removes the uncertainty of the positive result. If there is a negative result from a reliable and adequate Ames study, which removes the uncertainty of the Watanabe study then the *in vivo* study does not need to be performed. The test should be conducted using a suitable test material as indicated in Appendix 3, including in terms of purity and composition.

Having regard of a proposal for amendment submitted by a Member State Competent Authority concerning modifications to the experimental conditions of the comet assay, ECHA agrees that you may consider potential cross-linking properties of the registered substance in the experimental setup of the comet assay in order to detect crosslinks. Such a change in the experimental conditions would demand you to prepare and analyse two sets of slides: one set of slides submitted to the standard experimental conditions (as described in OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA crosslinks.

The modified experimental conditions may utilise one of the following options: (i) increase of electrophoresis time, e.g. as described reference 23 in the TG 489 ; (ii) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS) ; or (iii) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options ii and iii are described e.g. in references 36-39 in the TG 489 or Pant *et al.*, 2015). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

You are reminded that according to Annex X, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered".

In case you decide to perform the comet assay, you may consider examining gonadal cells when conducting the comet assay (OECD TG 489), as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. 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.

4. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(e) and 13(4) of the REACH Regulation, a technical dossier registered at [redacted] shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./ OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of Section 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA Guidance on information requirements and chemical safety assessment, chapter R.7a (version 6.0, July 2017), section R.7.6.

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information provided

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study records for two-generation reproductive toxicity studies (OECD TG 416) conducted with acrylic acid (CAS RN 79-10-7) and methyl acrylate (EC number 202-500-6).

ECHA notes that, according to Annex X, Section 8.7.3, column 2, two-generation reproductive toxicity studies (EU B.35/OECD TG 416) initiated before 13 march 2015 shall be considered appropriate to address this standard information requirement.

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

Hence, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

b) The specifications for the study design

Information from studies to be conducted before the extended one-generation reproductive toxicity study

The sub-chronic toxicity study shall be conducted before the extended one-generation reproductive toxicity study and the results from that study shall be used, among with other relevant information, to decide on the study design of the extended one-generation reproductive toxicity study following ECHA Guidance on information requirements and chemical safety assessment, chapter R.7a (version 6.0, July 2017), section R.7.6. The sub-chronic toxicity study may provide information on effects that is relevant for triggers (e.g. weight changes and histopathological observations of organs as indication(s) of one or more modes of action related to endocrine disruption which may meet the toxicity-trigger for extension of Cohort 1B or as evidence of specific mechanism/ modes of action and/or neurotoxicity and/or immunotoxicity which may meet the particular concern criteria for developmental neurotoxicity and/or developmental immunotoxicity cohorts).

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment*, chapter R.7a (version 6.0, July 2017), section R.7.6.

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a conducted range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Species and route selection

According to the test method EU B.56/ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment*, chapter R.7a (version 6.0, July 2017), section R.7.6.2.3.2. Since the substance to be tested is a liquid ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision and to the Member States' proposals for amendments, you expressed your intention to improve the weight of evidence by performing further toxicokinetic examinations and a new reproductive toxicity screening study according to OECD TG 422 with hydroxypropyl acrylate. You have provided toxicological summaries of reproductive toxicity studies performed with acrylic acid, methyl and butyl acrylate and propylene glycol. ECHA notes that some of the information was not present in the original submission and will be evaluated in the follow up process after it becomes available in the form of a dossier update.

c) Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method EU B.56./ OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

Currently, the extension of Cohort 1B and the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) are not requested. However, the sub-chronic toxicity study (90-day) requested in this decision (request *2*) and/or any other relevant information may trigger changes in the study design. Therefore, the sub-chronic toxicity study (90-day) is to be conducted first and the study results submitted to ECHA in a dossier update by ***3 January 2019***. If, on the basis of this update and/or other relevant information, a need for changes to the study design is identified, ECHA will inform you by ***25 March 2019*** (i.e. within three months after expiry of the 12-month deadline to provide the sub-chronic toxicity study (90-day)) of its intention to initiate a new decision making procedure under Articles 41, 50 and 51 of the REACH Regulation to address the design of the extended one-generation reproductive toxicity study. If you do not receive a communication from ECHA by ***25 March 2019***, the request of the present decision for the extended one-generation reproductive toxicity study remains effective and you may commence the conduct of the study and the results will need to be submitted by the deadline given in this decision ***25 March 2021***.

Notes for your considerationF

When submitting the study results of the sub-chronic toxicity study (90-day) you are invited to also include in the registration update your considerations whether changes in the study

design are needed (see also *ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7a* (version 6.0, July 2017), Section R.7.6.

Furthermore, after having commenced the extended one-generation reproduction toxicity study in accordance with the ECHA decision, you may also expand this study to address a concern identified during the conduct of it and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the changes in the study design must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/ triggers must be documented.

5. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(e) and 13(4) of the REACH Regulation, a technical dossier registered at [redacted] shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

Pre-natal developmental toxicity studies (test method EU B.31./ OECD TG 414) on two species are part of the standard information requirements for a substance registered for [redacted] (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier contains information on a pre-natal developmental toxicity study in rats by the inhalation route using the registered substance as test material.

For the second species, you have sought to adapt the information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study records for a *OECD Guideline 414 (Prenatal Developmental Toxicity Study)* and *Proposal for updating Guideline 414: Prenatal Developmental Toxicity Study (22 Jan 2001)* with the analogue substances acrylic acid (CAS RN 79-10-7) and methyl acrylate (EC number 202-500-6), respectively. Both studies were conducted using rabbit as the test animal.

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The test in the first species was carried out by using a rodent species (rats). According to the test method EU B.31/ OECD TG 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbits as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a (version 6.0, July 2017), section R.7.6.2.3.2. Since the substance to be tested is a liquid ECHA concludes that testing should be performed by the oral route.

In your comments, you have included toxicological summaries of reproductive toxicity studies in rabbits performed with acrylic acid, methyl and butyl acrylate and propylene glycol. ECHA notes that some of the information was not present in the original submission.

ECHA further notes that it will evaluate this information in the follow up process after it becomes available in the form of a dossier update.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species rabbits by the oral route.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 5 April 2016.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation:

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 proposal(s) for amendment.

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

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

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

In addition, you provided comments on the draft decision. These comments were not taken into account by the Member State Committee as they were considered to be outside of the scope of Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-54 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. The substance subject to the present decision is provisionally listed in the Community rolling action plan (CoRAP) for start of substance evaluation in 2019.

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

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

4. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the tests to be assessed.

5. In case the required tests are conducted with an analogue substance in the context of a read-across approach, the identity of the test material used to perform the test should be specified in line with ECHA's Practical Guide "*How to use alternatives to animal testing to fulfil the information requirements for REACH registration (chapter 4.4)*". This is required to demonstrate that the test material is representative of the analogue substance identified in the read-across approach and used to predict the properties of the registered substance.