ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 24 July 2019

Addressee: [redacted]

Decision number: CCH-D-2114476026-49-01/F
Substance name: 2-hydroxyethyl acrylate
EC number: 212-454-9
CAS number: 818-61-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 06.04.2016
Registered 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. **In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum with the registered substance.**

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

3. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: OECD TG 414) in a second species (rabbit), oral route with the registered substance.**

4. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce systemic toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity); and**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.**

5. **Robust study summary for the Carcinogenicity study (Annex X, Section 8.9.1. in conjunction with Annex I, Section 1.1.4.).**

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 to the REACH Regulation. 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 adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by ***31 January 2023*** except for the information requests for an *In vivo* mammalian alkaline comet assay (point 1, above); Sub-chronic toxicity study (90-day; point 2, above); Robust study summary for the Carcinogenicity study (point 5 above) which shall be submitted in an updated registration dossier by ***31 July 2020.*** You may only commence the Extended one-generation reproductive toxicity study as requested under point *4* after ***02 November 2020,*** 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 timeline has been set to allow for sequential testing.

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

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Authorised[1] by Ofelia Bercaru, Head of Unit, 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 1: Reasons

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 have adapted the standard information requirements for

- *In vivo* genotoxicity study (Annex IX, Section 8.4.);
- Pre-natal developmental toxicity study in a second species (Annex X, Section 8.7.2); and
- 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. You have also used a read-across approach as part of the adaptation for sub-chronic toxicity (90-days; Annex IX, Section 8.6.2).

Annex XI, Section 1.5. requires a structural similarity among the substances within a group or category such 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 an property-specific context.

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

You have summarised the read-across hypothesis as follows: *"The target chemical 2-hydroxyethyl acrylate (CAS no. 818-61-1) and its analogue chemicals hydroxypropyl acrylate (CAS no. 25584-83-2), 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 2-hydroxyethyl acrylate and hydroxypropyl acrylate contain an alcohol group which only differs in the length of the alcohol being either an ethyl or a propyl. 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."*

"[...] All substances are structural analogues with a similar functional group (acrylate). Furthermore, 2-hydroxyethyl acrylate, hydroxypropyl acrylate, and methyl acrylate will be rapidly metabolised by hydrolysis of the ester linkage by carboxylesterase to acrylic acid and ethylene glycol, propylene glycol, or methanol, respectively. In addition, the substances have similar physico-chemical and toxicological properties. As a results, the toxicity data derived from acrylic acid and the alcohols/glycols associated with the esters can be used to facilitate read-across to fill the few remaining data gaps and to support study results."

ECHA considers this as the hypothesis under which you make predictions for the properties listed above. ECHA understands that you predict *In vivo* genetic toxicity of the target (registered) substance, 2-hydroxyethyl acrylate (CAS No. 818-61-1; hereafter referred to as '**HEA**') from the analogue substance hydroxypropyl acrylate (CAS No. 25584-83-2; hereafter referred to as '**HPA**'). For Prenatal developmental toxicity, you predict the properties of HEA

from the source substances acrylic acid (CAS No. 79-10-7; hereafter referred to as '**AA**'), and methyl acrylate (CAS No. 96-33-3; hereafter referred to as hereafter referred to as '**MA**'). For toxicity to reproduction, you predict the properties of HEA from MA and AA.

Support of the grouping and read-across approach

You have provided a read-across justification as a separate attachment IUCLID Section 13. In summary you provide the following arguments to support the read-across approach:

Toxicokinetic information has been provided on HEA. The half-life of HEA *in vitro* in rat blood is approximately 100 seconds. *In vivo* absorption, distribution and excretion of uniformly radiolabelled HEA have been investigated in rats via oral, dermal and inhalation routes of exposure. There are no marked route-dependent differences in the metabolic fate of HEA. After 12 hours about 70% of the radiolabel was recovered in the urine (about 30%) as $^{14}CO_2$ (about 40%). In your read-across justification document, you present similar toxicokinetic conclusions for the source substance MA as for HEA above. Regarding HPA you state that *"similar metabolism for hydroxypropyl acrylate is to be expected* ", but no toxicokinetic information is provided. You conclude that "[...], *the toxicokinetic data shows that 2-hydroxyethyl acrylate, hydroxypropyl acrylate and methyl acrylate are rapidly metabolized to acrylic acid and their associated alcohols and glycols. As a results, the toxicity data derived from acrylic acid and the alcohols/glycols associated with the esters can be used to facilitates read-across to fill the few remaining data gaps and supports individual member study results."*

With regard to mutagenicity the following studies have been provided in IUCLID:

Mutagenicity in vitro:

i. Experimental study; Reliability 2 (reliable with restrictions); 1996; non-GLP; non-guideline (Principles of the test: "*A collaborative study of chemically-induced mutagenicity was performed using the four bacterial strains Salmonella typhimurium TA102 and TA2638 and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101 in order to compare the specific spectrum of response to chemicals among the four strains and to determine the usefulness (sensitivity) of each strain. Twenty laboratories participated in this study.*"); Study conducted with **HEA** in *S. typhimurium* strains TA 102, TA 2638 and *E. coli* WP2/pKM101 and WP2 uvrA/pKM101 up to 5000 µg/plate; Result is positive in *E. coli* WP2/pKM101 and WP2 uvrA/pKM101 with metabolic activation.

ii. Experimental study; Reliability 2 (reliable with restrictions); 1976; non-GLP; non-guideline (Principles of the test: "*For in vitro S. typhimurium assay test concentration used were 0.5, 1.0, 5, 10, 50, or 100 µg per plate for metabolic activation with and without.*"); Study conducted with **HEA** in *S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 up to 100 µg/plate; Result is negative with metabolic activation.

iii. Experimental study; Reliability 2 (reliable with restrictions); 1976; non-GLP; non-guideline (Principles of the test: "*For in vitro S. typhimurium assay test concentration used were 7500 nl per plate for metabolic activation with and without.*"); Study conducted with **HEA** in *S. typhimurium* strain TA 100 up to 7.5 µg/plate; Result is negative with metabolic activation.

iv. Experimental results; Reliability 2 (reliable with restrictions); 1989; non-GLP; non-guideline (Principles of the test: Gene mutation in mammalian cells; test conducted as described in Turner et al. (1984); study conducted with **HEA** up to 20 µg/ml; Result is positive without metabolic activation.

v. Experimental results; Reliability 2 (reliable with restrictions); 1989; non-GLP; non-guideline (Principles of the test: chromosome aberration study; test conducted as

described in Turner et al. (1984) and Doerr et al. (1989); study conducted with **HEA** up to 20 µg/ml; Result is positive without metabolic activation.

vi. Experimental results; Reliability 2 (reliable with restrictions); 1989; non-GLP; non-guideline (Principles of the test: *in vitro* micronucleus study; test conducted as described in Turner et al. (1984) and Doerr et al. (1989); study conducted with **HEA** up to 20 µg/ml; Result is positive without metabolic activation.

vii. Experimental results; Reliability 2 (reliable with restrictions); 1976; non-GLP; non-guideline (Principles of the test: in vitro DNA damage and/or repair in yeast); study conducted with **HEA** up to 0.1%; Result is negative.

Mutagenicity in vivo

viii. Read-across from supporting substance or analogue; Reliability 2 (reliable with restrictions); 2000; GLP; Mammalian Erythrocyte Micronucleus Test (OECD TG 474); test conducted in mice with **HPA** (600 mg/kg); results are negative,

ix. Experimental results; Reliability 2 (reliable with restrictions); 1979; non-GLP; non-guideline (Principles of the test: *"A chronic inhalation study in Sprague-Dawley rats included 12-month interim sacrifices for cytogenetic examinations. 4 male and 4 female rats per group were injected i.p. with colchicine (0.4 mg/kg bw), sacrificed 4 hours after injection and samples of bone marrow collected. Slides of the bone marrow were prepared for the microscopic examination of chromosomes. 50 cells per animal were scored for chromatid aberrations, chromosome aberrations and abnormal cells, with the exception of female controls where 35, 43, 19 and 25 cells were scored and one female in the 5 ppm group where only 2 cells were scored";* study conducted with **HEA** at 0.5 and 5 ppm; Result is negative.

Repeated dose toxicity

Studies via oral administration:

x. Experimental results; Reliability 2 (reliable with restrictions); 1967; non-GLP; non-Guideline (Principle of the test: *"Groups of male and female Sherman strain rats (10/sex/group) were maintained for 100 days on a diet containing 0, 0.03. 0.1 or 0.3 % HEA (equivalent to doses of approx. 0, 20, 65, and 196 mg/kg body weight/day for males and 0, 30, 102, and 305 mg/kg body weight/day for females)"*); study was conducted in rats with **HEA**; Results: NOAEL: 196/305 mg/kg/day (male/female; based on no effects);

xi. Experimental results; Reliability 2 (reliable with restrictions); 1967; non-GLP; non-Guideline (Principle of the test: *"Groups of male and female Beagle dogs (2/sex/group) were maintained for 97 days on a diet containing 0.06, 0.2, or 0.4 % HEA in diet (equivalent to doses of 21, 60 and 125 and 22, 63 and 131 mg/kg body weight/day for males and females respectively)"*); study was conducted in dogs with **HEA**; Results: NOAEL 125/131 mg/kg/day (male/female; based on no effects);

Studies via inhalation administration:

xii. Experimental results; Reliability 2 (reliable with restrictions); 1970; non-GLP; non-Guideline (Principle of the test: "Three groups of animals, each consisting of 25 male Sherman rats, were exposed to 5, 10, and 25 ppm of HEA vapours, respectively. The duration of exposures was seven hours per day, five days per week for a total of twenty exposures"); study was conducted in rats with **HEA**; Results: LOAEC 5 ppm (based on severe local irritation); and

xiii. Experimental results; Reliability 2 (reliable with restrictions); 1979; non-GLP; non-Guideline (Principle of the test: combined repeated dose and carcinogenicity "Groups of 99-100 male and female rats were exposed to atmospheres containing 0 ppm (controls), 5.0 ppm (24 mg/m3) or 0.5 ppm (2.4 mg/m3) 2-hydroxyethyl acrylate (HEA) for 6 hours/day, 5 days/week over an 18 month period, and subsequently held

for a post-exposure period of 5 months (males) and 6 months (females)."); study was conducted in rats with **HEA**; Results: NOAEC 0.0024 mg/L air (based on: "Increased incidence, increased severity, and earlier onset of the lesions associated with chronic murine pneumonia at the high dose level.").

xiv. Read-across from supporting substance; Reliability 2 (reliable with restrictions); 1983; non-GLP; non-Guideline (Principles of the test: *'Three groups of animals, each consisting of 2 male beagle dogs, 4 male New Zealand white rabbits, 10 male Sprague-Dawley rats (Spartan strain) and 20 Swiss-Webster male mice were used in this study. Two groups of animals were designated to be exposed to HPA vapours of 5 ppm (0.027 mg/L) or 10 (0.053 mg/L), respectively. The duration of exposure was 6 hours per day, 5 days per week for a total of 20 exposures for dogs and rabbits and 21 exposures for rats and mice in 30-31 days. The third group served as an unexposed control group.'*; study conducted using **HPA** administered via inhalation to males only; Results: $LOAEC_{rat}$ 5 ppm (based on local effects in the upper respiratory system); $NOAEC_{mouse}$ 5 ppm (based on bodyweight and eye irritation); $LOAEC_{dog}$ 5 ppm (based on local effects in the upper respiratory system); and $LOAEC_{rabbit}$ 5 ppm (based on local effects in the upper respiratory system);

Fertility and developmental toxicity

xv. Key study; read-across from supporting substance; Reliability 1 (reliable without restrictions); 2009; GLP; according to OECD TG 416; study conducted in rats using **MA** administered via inhalation; Doses: 0, 5, 25 and 75 ppm; Results: NOEC (P) 25 ppm (based on reduced body weight); NOAEC (P) fertility 75 ppm (highest dose tested); NOEC (F1) 25 ppm (based on reduced pup weight);

xvi. Key study; read-across from supporting substance; Reliability 1 (reliable without restrictions); 1994; GLP; according to OECD TG 416; study conducted in rats using **AA** administered via drinking water; Doses: 0, 53, 240 and 460 mg/kg/day; Results: NOAEL (P) toxicity 240 ppm (based on general toxicity); NOAEL fertility 460 mg/kg/day (highest dose tested); NOAEL (F1) 460 mg/kg/day;

xvii. Experimental results; Reliability 2 (reliable with restrictions); 1999; non-GLP; non-guideline (Principles of the test: "Groups of 20-29 bred female rats (17-25 pregnant) were exposed to the compound 6h/day on days 6 through 20 of gestation by inhalation. Control animals were exposed concurrently to filtered room air. The test concentrations of 2-hydroxyethyl acrylate were 1, 5, and 10 ppm (corresponding to approx. 4.8, 24.1, and 48.2 µg/L)"; test conducted in rats with **HEA** administered via inhalation; Results: NOAEC maternal toxicity 5 ppm (based on body weight gain); NOAEC developmental toxicity 10 ppm (highest does tested);

xviii. Read-across from supporting substance; Reliability 1 (reliable without restrictions); 2009; GLP; according to OECD TG 414; test conducted in rabbit with **MA** administered via inhalation; Doses tested; 0, 5, 15 and 45 ppm; NOAEC maternal toxicity 15 ppm (based on local effects in the nasal cavity); NOAEC developmental toxicity 45 ppm (highest does tested).

xix. Read-across from supporting substance; Reliability 2 (reliable with restrictions); 1993; GLP; similar to OECD TG 414 (Deviations: N=16 per group); test conducted in rabbit with **AA** administered via inhalation; Doses tested; 0, 25, 75 and 225 ppm; NOAEC maternal toxicity 25 ppm (based on local effects in the nasal cavity); NOAEC developmental toxicity 225 ppm (highest does tested).

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

According to ECHA's understanding, you suggest that predictions of the properties of the target substance is possible because:

(i) all substances are stucturally similar, *i.e.* they are all esters of acrylic acid with similar functional group (acrylate) and "*where applicable, the alcohol group*";
(ii) "*the substances have similar physico-chemical and toxicological properties*";
(iii) *The acrylate esters have been shown to be metabolized in the mammalian body in minutes to acrylic acid and the corresponding alcohol.*

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

(i) Structural (dis)similarities and their impact on the prediction

Structural similarity is a prerequisite for applying the grouping and read-across approach, but ECHA does not accept in general or this specific case that structural similarity *per se* is sufficient to enable the prediction of human health properties of a substance, since structural similarity does not always lead to predictable or similar human health properties. 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.

Furthermore, ECHA notes that you have clearly identified the structural similarities between the substances (*i.e.* the acrylic acid modality), and state that *'The difference in length of the backbone is toxicologically of lesser importance.' 'Except for propylene glycol, harmonized classifications exist for all metabolites. In addition, ECHA disseminated dossiers are available for all metabolites. None of the harmonized classification and ECHA disseminated dossiers indicate that the metabolites are genotoxic, carcinogenic, or reprotoxic.'* However, you have not provided information on these substances in your registration dossier.

ECHA concludes that no information has been provided on 1,2-ethanediol; 1,2-propanediol; or methanol. The provided explanation is therefore not sufficient to establish a scientifically credible link between the structural similarity and the prediction.

(ii) Similar physicochemical and toxicological properties

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances". One prerequisite for a prediction based on read-across therefore is that the substances involved are structurally similar and are likely to have similar properties. One important aspect in this regard is the analysis of the data matrix to compare the properties of source and target substances and to establish whether indeed they are similar or follow a regular pattern.

You state that "*the substances have similar physico-chemical [...] properties*"; and that the physico-chemical parameters/properties of target and source substances are similar. You propose that the similar physico-chemical properties of the target and source substances support the read-across between the substances.

ECHA observes that the physico-chemical properties of target and source substances are in the same/similar range.

You state that "*the substances have similar [...] toxicological properties*" and state that it is possible to predict the properties of HEA from HPA, MA, and AA because these substances have based on similar functional groups. Furthermore, you state that the toxicity of the substances "*on most of the tested endpoints are comparable*". ECHA notes that the data matrix provided on Table 1 of the read-across justification document supports this claim for

acute toxicity, skin and eye irritation and sensitisation. However, for the endpoints listed below ECHA notes differences between the source and target substances, or there is not enough information to confirm the validity of the read-across approach.

Genetic toxicity

With regard to "mutagenicity", you have in your read-across justification document compared the results of the *in vitro* and *in vivo* mutagenicity between the target substance HEA and the source substance HPA (no endpoint study summaries have been provided in IUCLID).
With regard to *in vitro* mutagenicity, ECHA notes that HEA is:

- POSITIVE for gene mutations in *E. coli* strains strain WP2/pKM101 and WP2 uvrA/pKM101 with metabolic activation (see study i above);
- POSITIVE for gene mutations in mammalian cells without metabolic activation (see study iv above); and
- POSITIVE for chromosomal aberrations in mammalian cells without metabolic activation (see study v and vi above).

ECHA notes that with regard to *in vitro* mutagenicity HPA is:

- POSITIVE for gene mutations in *E. coli* strains strain WP2/pKM101 and WP2 uvrA/pKM101 with or without metabolic activation;
- NEGATIVE for gene mutations in mammalian cells with or without metabolic activation; and
- POSITIVE for chromosomal aberrations in mammalian cells without metabolic activation.

ECHA concludes that the *in vitro* mutagenicity properties of HEA and HPA differ with regard to gene mutations in mammalian cells.

With regard to *in vivo* mutagenicity, ECHA notes that:

- HEA did not induce chromosomal aberrations (see study ix above), however, ECHA considers that this study is inconclusive due to too few metaphases counted in the study (see endpoint request 1 below).
- HPA is NEGATIVE chromosomal aberrations (see study viii above).

ECHA considers that due to shortcomings in the *in vivo* chromosomal aberration study with HEA, a similar *in vivo* genotoxicity profile between HEA and HPA it cannot be verified. In addition, no genotoxicity data has been provided on the non-common metabolites.

ECHA concludes that due to differences in the *in vitro* mutagenicity between HEA and HPA and inadequate *in vivo* study with HEA, similar genotoxicity profile of the substances cannot be verified.

Repeated dose toxicity

With regard to oral repeated dose toxicity, you have in the two studies on HEA (see studies x and xi above). No information with regard to oral repeated dose toxicity has been provided on the source substances HPA, AA or MA. The deficiencies of the oral studies on HEA are discussed in in the endpoint request 2 below.

With regard to inhalation repeated dose toxicity, you have in the read-cross justification document compared the relative toxicity of the AA and MA with that of HEA (see Table 1 of the read-across justification document).
ECHA notes that both the systemic local effects differ significantly between HEA and the proposed source substances:

- $NOAEC_{systemic\ effects}$ with HEA >24 mg/m^3 compared to a $NOAEC_{systemic\ effects}$ with AA 221 mg/m^3; and a $NOAEC_{systemic\ effects}$ with MA >519 mg/m^3; and

- $NOAEC_{local\ effects}$ with HEA 2.4 mg/m^3 compared to a $NOAEC_{local\ effects}$ with AA 74 mg/m^3; and a $LOAEC_{local\ effects}$ with MA 58 mg/m^3.

ECHA notes that the predominant effect observed in the repeated does toxicity studies are local effects, however these effects occur at quite different dose levels. Despite the concerns raised by ECHA with regard to the reporting of the carcinogenicity study with HEA (see request 5) this substance seems to be the most potent with regard to local effects following inhalation exposure. With regard to systemic effects, it should be noted that the available evidence does not support a claim of similar toxicity, as the no effects levels differ significantly between the source and target substances. You have not explained how the properties of HEA can be predicted from what appears to be less toxic substances.
In addition to the studies listed above, a sub-acute study with HPA was provided (see study xii above). However, the duration of this study is not sufficient to meet the requirements of a sub-chronic toxicity study (90-days).

Fertility and developmental toxicity

With regard to pre-natal developmental toxicity, ECHA notes that you have provided a pre-natal developmental study in rats on HEA (see study xvii) and pre-natal developmental studies in rabbit for AA (see study xix above) and MA (see study xviii above). ECHA notes that the dose selections vary between these studies: the dose ranges are 1-10 ppm for HEA (rat), 5-45 ppm for MA (rabbit), and 25-225 ppm for AA (rabbit). ECHA notes that the source and the target studies have been conducted in different species, therefore a reliable comparison between the source and target substances is not possible. In the absence of the comparable information in the same species, ECHA cannot assess your claim of absence of teratogenicity.

With regard to reproductive toxicity, you have provided Two-generation reproductive toxicity studies on MA (see study xv) and AA (see study xvi). ECHA notes that no information has been provided for HEA. Without any data on reproductive toxicity for HEA, a comparison of the reproductive toxicity profile of HEA and the source substances is not possible.

Non-common metabolites

ECHA understands that in addition to structural similarity and similar functional groups, your hypothesis is based on similar metabolism resulting in formation of a common metabolite acrylic acid and non-common metabolites ethylene glycol (from HEA), propylene glycol (from HPA) and methanol (from MA). In your read-across justification document, you mention that "*Besides the source chemical acrylic acid (CAS No. 79-10-7), the other metabolites of 2-hydroxyethyl acrylate, hydroxypropyl acrylate and methyl acrylate are methanol (CAS No. 67-56-1), ethylene glycol (CAS No. 107-21-1), and propylene glycol (CAS No. 57-55-6). Except for propylene glycol, harmonized classifications exist for all metabolites. In addition, ECHA disseminated dossiers are available for all metabolites. None of the harmonized classification and ECHA disseminated dossiers indicate that the metabolites are genotoxic, carcinogenic, or reprotoxic.*". ECHA notes that data on the source substances has not been provided in your registration dossier. ECHA considers that this information is essential in supporting the read-across approach.

ECHA also stresses that the information on the analogue substance MA provided in your adaptation, as currently documented, does not address the potential impact of exposure to 1,2-ethandiol formed from the target substance HEA, *i.e.* in conjunction with exposure to the other primary metabolite acrylic acid, on the toxicological properties of HEA. For all these reasons, ECHA considers that your read-across adaptation, as currently documented, does not constitute a reliable basis for predicting the properties of the registered substance according to the provisions of Annex XI, Section 1.5 of the REACH Regulation.

ECHA concludes that based on the presented evidence it is not possible to conclude on a similar or regular pattern of toxicity as a result of structural similarity. Furthermore, the non-common hydrolysis products have not been considered in the prediction. Therefore, it cannot be verified that the proposed analogue substances can be used to predict properties of the registered 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.

The *in vivo* studies conducted with HEA via the oral, dermal, inhalation and intra-peritoneal routes provide reliable information on the metabolism, distribution and elimination of HEA after unique administration to rat. These studies provide global toxicokinetic information integrating all the steps of the metabolic pathway of HEA, *i.e.* from the ester hydrolysis, the formation of the primary metabolites ethylene glycol (*i.e.* 1,2-ethanediol) and acrylic acid and until the elimination of the final metabolites. However, in the context of this specific weight of evidence approach, the characterization of the kinetics of the ester function hydrolysis and the determination of the formation of acrylic acid and the related alcohol constitutes the most relevant metabolic step. The uniform radio-labelling of the test material prevents from discriminating the formation of the primary metabolites formed, i.e. acrylic acid and ethylene glycol and the global study design does not specifically inform on the kinetics of the metabolic step of interest, *i.e.* the hydrolysis of the ester function. Further, no information from similar studies conducted with the target substance is available, allowing for a direct comparison of the toxicokinetic parameters derived from these studies between HPA and MA. Therefore, ECHA considers that the relevance of the information obtained from these *in vivo* toxicokinetic studies in the context of this read-across approach is not established. Furthermore, in your read-across justification document you shortly argue similar toxicokinetic properties of MA, however, no toxicokinetic data is provided in the technical dossier. Moreover, no toxicokinetic data has been provided on the hydrolysis rate of HPA.

Although information establishing a rapid metabolism of HEA is provided and whilst the general claim of hydrolysis of esters by carboxyl esterases is considered plausible, ECHA emphasises that your adaptation, as currently documented, is missing scientific evidence supporting your assumption that the source substances involved in this read-across approach, and in particular that the source substances HPA and MA, will display kinetics similar to those of documented and characterised in your dossier for HEA.

Consequently, it is not possible to conclude whether there are differences in the toxicokinetic behaviour, in particular in metabolic fate / (bio)transformation of the substances and how these differences may influence the toxicity profile of the target and source substances. ECHA considers that based on the lack of toxicokinetic data, there is not an adequate basis for predicting the properties of HEA from the data of the source substances.

(iv) Bias in the selection of source substances and source study

ECHA notes that you have not considered all available information relevant for the prediction. Firstly, there is a Pre-natal developmental toxicity study available for HPA, which has not been considered for the prediction. Secondly, as you have mentioned in the read-across justification document, there is information available about the toxicity of the non-common hydrolysis

products (methanol, propylene glycol and ethylene glycol). However, this data has not been provided in your registration dossier.

ECHA concludes that it is not possible to verify that you have selected the source substances which are most appropriate and furthermore that the source studies selected are giving rise to the highest concern as required in Annex I, Section 1.1.4.

Conclusion on the read-across approach

The adaptation of the standard information requirements for the endpoints *In vivo* genotoxicity study (Annex IX, Section 8.4.); Sub-chronic toxicity (90-days; Annex Xi, Section 8.6.2);Pre-natal developmental toxicity study in a second species (Annex X, Section 8.7.2); and Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3) 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. Thus, 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.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than [redacted] per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant."

The technical dossier contains several *in vitro* studies with HEA investigating gene mutation in bacteria (see study i-iii above), gene mutations in mammalian cells (see study iv above), and chromosome aberrations in mammalian cells (see study v and vi above) which all show positive results. The positive results indicate that the substance is inducing gene mutations and chromosomal aberrations under the conditions of the tests.

The technical dossier contains an *in vivo* study a non-guideline, pre-GLP chromosome aberration study that shows negative results (see study ix above). While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex XI, Section 1.1.2. According to Article 13(3) of the REACH Regulation, tests required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods recognised by the Commission or ECHA.

Other tests may be used if the conditions of Annex XI are met. More specifically, Section 1.1.2. of Annex XI provides that existing data on human health properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) shall be considered equivalent to data generated by the corresponding test methods referred to in Article 13(3) if the following conditions are met:

(1) Adequacy for the purpose of classification and labelling and/or risk assessment;
(2) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
(3) Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and
(4) Adequate and reliable documentation of the study is provided.

With regard to the pre-GLP chromosome aberration study with HEA, ECHA notes that the methodology used in this study deviates significantly from the current OECD TG 474. ECHA notes the following major deviations: the number of cells scored is 2-50 per animal, while the OECD TG 474 requires that "*at least 4000 immature erythrocytes per animal should be scored for the incidence of micronucleated immature erythrocytes*"; there is no historical control data; and there is no proof that the substance reaches the bone marrow. ECHA concludes that is study is not reliable and cannot be used to even partially meet the "mutagenicity" standard information requirements or support read-across.

In addition, you have provided an *in vivo* micronucleus test with the source substance HPA (study viii above). However, as explained above, your read-across adaptation is rejected thus this study cannot be used to (partially) fulfill the standard information requirements with regard to in vivo mutagenicity Annexes IX and X, Section 8.4.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and chromosomal aberrations is not available for the registered substance. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("Comet Assay", OECD TG 489) is suitable to follow up positive *in vitro* results for gene mutation and chromosomal aberrations. Hence, ECHA considers this test to be the most appropriate for the substance subject to the decision.

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 OECD TG 489, the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to 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 on the initial draft decision you disagree with the request. You have conducted a new OECD TG 471 assay with HEA and HPA and a new OECD TG 476 test on HEA. Based on these tests you argue that HEA should be considered negative for gene mutation in both bacteria and mammalian cells. With regard to chromosome aberration you acknowledge that the available *in vitro* test is positive. You also do not question ECHA's reasoning that the available in vivo study on HEA is not adequate to address the concern; instead you argue that the results from the available carcinogenicity study and the *in vivo* chromosome aberration combined indicate that the substance is not a clastogen. You also

propose to cover this information requirement by reading across from HPA, MA and n-butyl acrylate (nBA).

With regard to the newly generated information, ECHA is unable to assess how this information would influence the overall weight of evidence for each of the three mutagenicity tests because the robust study summaries of the results have not been provided. In addition you have not provided a detailed reasoning on how you reach an overall conclusion for each of the tests. In general, all available information for each of the tests should be considered using weight of evidence; this needs to be clearly reported. Currently due to poor reporting ECHA is unable to assess the carcinogenicity study on HEA (see issue 5). Based on the information provided it is therefore currently not possible for ECHA to reach a conclusion on your proposed read-across approach. It should in this context be highlighted that when read-across is used all results of a study are read across from the source substance to the target substance. You propose to read across from multiple source substances but do not explain how you intend to do this prediction.

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: *In vivo* mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

Notes for your consideration

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". You may consider examining gonadal cells in addition to the other aforementioned tissues, 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.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than [redacted] per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same 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.

In the technical dossier you have provided the following study records for sub-chronic toxicity studies in rats and dogs via the oral route with HEA (see studies x and xi above). In addition, you have also provided a sub-acute and a chronic toxicity study via the inhalation route with HEA, which are reported in IUCLID as key studies (see studies xiii and xiv above), and a sub-acute study conducted with HPA (see study xii above).

According to Article 13(3) of the REACH Regulation, tests required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods recognised by the Commission or ECHA.

Other tests may be used if the conditions of Annex XI are met. More specifically, Section 1.1.2. of Annex XI provides that existing data on human health properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) shall be considered equivalent to data generated by the corresponding test methods referred to in Article 13(3) if the following conditions are met:

(1) Adequacy for the purpose of classification and labelling and/or risk assessment;
(2) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
(3) Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and
(4) Adequate and reliable documentation of the study is provided.

With regard to the studies via the oral route of administration, ECHA notes that the information provided in the endpoint study records does not fulfil the conditions described in the current version of the OECD TG 408 or OECD TG 409. Based on the information reported in the robust study summaries provided for these studies, ECHA is unable to fully understand how these studies deviate from the current test guideline. It should be noted that the animal numbers in the dog study is half of those required by the OECD TG 409; i.e. at least 4 animals per sex per group. Furthermore, according to the recommendations of the OECD TGs, the "*highest dose should be chosen with the aim to induce toxicity but not death or severe suffering*". In these studies, no toxicity was observed up to the highest dose tested, leading to the identification of NOAELs corresponding to the highest test doses tested in the studies.

With regard to the studies via the inhalation route of administration, ECHA notes that the reporting detail of the chronic toxicity study is not sufficient to allow and independent assessment of the results or the method used (for details on missing elements see request 5 below). The sub-acute study is not of a comparable duration to a sub-chronic study and consequently not sufficient to meet the standard information requirement of Annex IX, Section 8.6.2.

In IUCLID you have flagged the studies via oral route as weight of evidence but you have not explained why these two studies constitute a weight of evidence with regard to oral sub-chronic toxicity. Thus, the weight of evidence cannot be assessed.

ECHA concludes that none of the studies provided meet the conditions of Annex XI, Section 1.1.2 for use of old data nor do they jointly meet the conditions of a weight of evidence of Annex XI, Section 1.2.

According to Annex IX, Section 8.6.2. Column 2, a sub-chronic toxicity study (90 days) does not need to be conducted, if a reliable chronic toxicity study in an appropriate species and route of administration is used. However, in this case ECHA is unable to verify the reliability of the chronic toxicity study (see request 5 below). Finally ECHA notes that you are not using the chronic toxicity study (study xiii above) for DNEL derivation; instead you are using the results of the sub-acute study (study xii above).

You have also provided a supporting sub-acute toxicity studies on HPA in rats, mice, rabbits and dogs. A sub-acute study is not of a comparable duration to a sub-chronic study and consequently not sufficient to meet the standard information requirement of Annex IX,

Section 8.6.2. In addition, as explained above, your read-across adaptation is rejected for systemic toxicity endpoints.

Therefore, your adaptations of the information requirement is rejected.

Consequently, there is an information gap and it is necessary to provide information for this endpoint.

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* (version 6.0, July 2017) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. More specifically, the substance is a liquid of 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 OECD TG 408.

According to the test method *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 on the initial draft decision you disagree with the request and express your intention to provide a refined weight of evidence assessment for this endpoint considering all available information on HEA, HPA, MA and n-butyl acrylate (nBA) and the HEA's hydrolysis products AA and ethane-1,2-diol (ethylene glycol; EG).

In the revised read-across justification document, you list the effects observed in studies conducted with on HEA, HPA, MA, nBA, AA and EG. You then conclude "*Based on the absence of systemic effects in the available sub-chronic oral studies and inhalation studies (section 3.2.5) HEA is not expected to be toxic after repeated dose exposure via the oral route. This is supported by the data on HPA, nBA, MA and AA since these substances are not classified for repeated dose toxicity. The HEA metabolite ethane-1,2-diol has a STOT RE Cat. 2 classification based on adverse kidney effects relate to oxalate accumulation. Since no comparable effects are observed in the studies with HEA up to the highest dose tested. In addition for ethane-1,2-diol these effects are not prevalent at doses below 150 mg/kg bw/day. Based on the maximum ethane-1,2-diol release from HEA this would translate to an effect level of approximately 280 mg/kg bw/day for HEA (based on a mw of ethane-1,2-diol of 62.07 g/mol). In addition, human are expected to have higher clearance rates for oxalate crystals, making the effects in humans less severe. Altogether the adaption of a STOT-RE classification for HEA seems inappropriate.*"
ECHA disagrees with this conclusion for several reasons.

Firstly, ECHA has requested the study to be conducted via the oral route therefore your read-across/weight of evidence adaptation should cover predictions for this route of exposure.

Secondly, as stated above, ECHA is unable to fully understand how the available information on HEA deviate from the current test guideline. Without a clear picture of what was measured and what was not measured in these studies it is impossible to assess how much support is gained to the proposed read-across that is provided by the existing studies on HEA.

Thirdly, ECHA considers the results on HEA (despite the limitations highlighted above) inconsistent with the results of some of your source studies. There seem to be significant different effects observed depending on whether the substance has been administered in the

diet/drinking water or via oral gavage. HEA shows no effects in rats after 100 days up to 196-305 mg/kg/day (in diet). MA has no effects up to 20 mg/kg/day (highest dose tested in drinking water) in a 90 day study. nBA shows no effects in rats after 90 days up to 84/111 mg/kg/day (M/F; in drinking water); and the hydrolysis product of HEA, i.e. AA , shows no adverse effect after 12 months at 40/375 mg/kg/day (M/F). In contrast, HPA has a NOAEL of 15 mg/kg/day based on gastrointestinal effects in an OECD TG 422 study (oral gavage); the hydrolysis product of HEA, i.e. AA shows GI effects and adverse renal effects at 150 mg/kg/day after 90 days; and the other hydrolysis product of HEA, i.e. EG, is classified as STOT RE Cat. 2, H373 for renal effects via the oral route.

Fourthly, the studies above with HEA, MA, nBA via the oral route are not dosed high enough to detect potential hazards for sub-chronic toxicity (the limit dose is 1000 mg/kg/day as required in the OECD TG 408); absence of effects in studies conducted below the limit dose does not support a robust conclusion of absence of toxicity.

Fifthly, for hazard identification the studies giving rise to the highest concern should be used (Annex I, Section 1.1.4). In this case the studies via oral gavage are those giving rise to the highest concern. These studies show toxicity and therefore your conclusion on absence of systemic effects is not supported. Finally, Annex XI, Section 1.5. require that whenever read-across is used the substance should be classified on this basis.

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: OECD TG 408) in rats.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than [redacted] per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Pre-natal developmental toxicity studies (test method OECD TG 414) on two species are part of the standard information requirements for a substance registered for [redacted] or more per year (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).

You have sought to adapt the information requirement for a pre-natal developmental toxicity study in a second species according to Annex XI, Section 1.5. of the REACH Regulation by providing study records for a Pre-natal developmental toxicity (OECD TG 414) in rabbits with the source substances AA and MA. However, as explained above, your adaptation of the information requirement is rejected.

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 (rat). According to the test method OECD 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 rabbit 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* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.2.2. 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 initial draft decision you disagree with the request and maintain your position that read-across is possible. Furthermore, you highlight that there is newly generated information available for two of the source substances HPA and nBA. In addition you have added available information in rats and rabbits for your source substances. Your read-across justification document now identifies available information on prenatal developmental toxicity in rats, rabbits and mice for HEA, MA, nBA and the hydrolysis products AA and EA. The combined results indicates that none of the source substances are developmental toxicants in rats or rabbits and that read-across may be plausible.

However, based on your comments ECHA is unable to reach a firm conclusion because the information provided in your comments is not detailed enough to support a conclusion.

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: OECD TG 414) in a second species (rabbit) by the oral route.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than [redacted] per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

The basic test design of an extended one-generation reproductive toxicity study (test method 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 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, Section R.7.6 (version 6.0, July 2017).

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 a study record for a Two-generation reproductive toxicity study (OECD TG 416) with the source substance MA and AA. However, as explained above, your adaptation of the information requirement is rejected. 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 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, Section R.7.6 (version 6.0, July 2017).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, Section R.7.6 (version 6.0, July 2017).

The highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and 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 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* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.2.3. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

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 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 systemic 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.

In your comments on the initial draft decision you disagree with the request. You highlight that there is an additional source study available (an EOGRTS on nBA) and an OECD TG 422 on HPA. You have also added OECD TG 416 studies of MA and AA and a 3-generation study on EG. The results of these studies appear consistent but no firm conclusion can be reached based on the available information because there is no information on fertility and reproductive function available on the registered substance (HEA) to compare the toxicity profile against.

You argue that the OECD TG 422 study on HPA provides reliable screening level results for this endpoint. ECHA agrees that an OECD TG 422 study provides screening level information which substantially strengthens a read across approach. However, screening level information is not available for HEA. If such a study (OECD TG 422) was available it would provide screening level information on repeated dose toxicity and reproductive/developmental toxicity which could be used to compare the toxicity profile of the target substance with that of the source substance. Without such information, the support for read-across for this endpoint is currently weak.

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 ***31 July 2020***. 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 ***02 November 2020*** (*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 ***02 November 2020***, 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 ***31 January 2023***.

Notes for your consideration

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, Section R.7.6 (*version 6.0, July 2017*)*).

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. Robust study summary for the "Chronic toxicity/Carcinogenicity study" (Annex X, Section 8.9.1. in conjunction with Annex I, Section 1.1.4.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than [redacted] per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

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. Guidance on the preparation of the robust study summaries is provided in the Practical Guide on "How to report robust study summaries".

Furthermore, pursuant to Article 10 (a)(vii) and Annex I, Section 1.1.4. if there are several studies addressing the same effect, then, having taken into account possible variables (e.g. conduct, adequacy, relevance of test species, quality of results, etc.), normally the study or studies giving rise to the highest concern shall be used to establish the DNELs and a robust study summary shall be prepared for that study or studies and included as part of the technical dossier. Robust summaries will be required of all key data used in the hazard assessment.

You have provided a study record for a "Chronic toxicity/Carcinogenicity study" ([redacted] 1979. This study reports "*Neoplasms, all of which were considered spontaneous in origin, occurred in the following organs and tissues: liver, lung, pancreas, kidney, testes, preputial gland, stomach, small and large intestine, spinal cord and peripheral nerves, pituitary gland, subcutaneous tissue, mammary tissue, integument, ear canal, oral cavity, adrenal gland, lymph nodes, spleen, brain, adipose tissue, thyroid, ovary, uterus, clitoral gland and vagina.*"

However, ECHA notes that, contrary to Article 3(28) of the REACH Regulation, the documentation of this study is insufficient and does not allow an independent assessment of the adequacy of this study, its results and its use for hazard assessment.

In particular, the following elements are missing:

- A clear description as to how this study compares to a Combined Chronic Toxicity/Carcinogenicity Study according to OECD TG 453 in terms of study design and parameters investigated.
- Results tables for body weights, organ weights and their ratios (if applicable), Necropsy findings; Incidence and severity of abnormalities.
- Detailed report on Histopathology (Non neoplastic histopathological findings; Neoplastic histopathological findings; Correlation between gross and microscopic findings; Detailed description of all treatment-related histopathological findings

including incidence and severity gradings).
- Historical control data.
- Consideration of any mode of action information
- Therefore, you need to provide a complete robust study summary with the above missing elements for this study.

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.
In your comments you agreed to the request.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information: Robust study summary for the "Chronic toxicity/Carcinogenicity study" ([redacted] 1979).

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 20 February 2017.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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 proposals for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

You provided comments only on the draft decision. Your 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 in its MSC-65 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

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

3. 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 grades registered to enable the relevance of the tests to be assessed.