EUROPEAN CHEMICALS AGENCY

Helsinki, 15 July 2015

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DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For Butyl acrylate, CAS No 141-32-2 (EC No 205-480-7)

Addressees: Registrant(s)[1] of Butyl acrylate (Registrant(s))

This decision is addressed to all Registrants of the above substance with active registrations on the date on which the draft for the decision was first sent for comment, with the exception of the cases listed in the following paragraph. A list of all the relevant registration numbers subject to this decision is provided as an annex to this decision

Registrants holding active registrations on the day the draft decision was sent are *not* addressees of this decision if they are: i) Registrant(s) who had on that day registered the above substance exclusively as an on-site isolated intermediate under strictly controlled conditions and ii) Registrant(s) who have ceased manufacture/import of the above substance in accordance with Article 50(3) of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation) before the decision is adopted by ECHA.

Based on an evaluation by the Swedish Chemicals Agency as the Competent Authority of Sweden (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 29 April 2014, i.e. the day on which the draft decision was notified to the Registrant(s) pursuant to Article 50(1) of the REACH Regulation. Although the evaluating MSCA did not grant an extension for submitting dossier updates which it would take into consideration, the evaluating MSCA received an updated justification for the acrylate category which was taken into account.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Sweden has initiated substance evaluation for butyl acrylate CAS No 141-32-2 (EC No 205-480-7) based

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Human health/Suspected reproductive and developmental toxicity; Exposure/Occupational exposure; Aggregated tonnage, butyl acrylate was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013. The updated CoRAP was published on the ECHA website on 20 March 2013. The Competent Authority of Sweden was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA noted additional concerns regarding potential mutagenicity of the substance and derivation of DNEL.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 20 March 2014.

On 29 April 2014 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant commenting phase

By 5 June 2014 ECHA received comments from concerned registrants of which it informed the evaluating MSCA without delay on 11 June 2014.

The evaluating MSCA considered the comments received from the Registrant(s). The information contained therein is reflected in the Statement of Reasons (Section III), where appropriate, whereas no amendments to the Information Required (Section II) were made.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 15 January 2015 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, four Competent Authorities of the Member States and ECHA submitted proposals for amendment (PfAs) to the draft decision.

On 20 February 2015 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment received and amended sections II and III of the draft decision.

Referral to Member State Committee

On 2 March 2015 ECHA referred the draft decision to the Member State Committee.

By 23 March 2015 in accordance to Article 51(5), the Registrant(s) provided comments on the proposals for amendment. In addition, the Registrant(s) provided comments on the draft decision. The Member State Committee took the comments on the proposal(s) for amendment of the Registrant(s) into account. The Member State Committee did not take into account the Registrant(s) comments on the draft decision as they were not related to the proposal(s) for amendment made and are therefore considered outside the scope of Article 51(5).

After discussion in the Member State Committee meeting on 20 to 23 April 2015, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 23 April 2015. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods (in accordance with Article 13 (3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

1. **Extended one-generation reproductive toxicity study in rats, via the oral route (test method: EU B.56./OECD 443) without the Cohorts 2 and 3 and without the extension of Cohort 1B to mate the F1 animals to produce the F2 generation.**

   The premating exposure shall be 10 weeks and the highest dose should be selected with the aim to induce some toxicity.

2. **Prenatal developmental toxicity study in rabbits, via the oral route (test method: EU B.31./OECD 414)**

3. **Tiered approach strategy for mutagenicity potential assessment (Tiers 1-3)**

   Tier 1:

   In vitro mammalian cell micronucleus test (test method: EU B.49./OECD 487) with the addition of a chromosome centromere labelling method (e.g. FISH, CREST)

   Tier 2:

   a) **In case of a negative result in In vitro mammalian cell micronucleus test: the In vitro mammalian cell gene mutation test using the thymidine kinase gene (test method: new test guideline OECD 492)[2];**
   b) **In case of positive result in the In vitro mammalian cell micronucleus test mainly demonstrating aneuploidy: the Mammalian erythrocyte micronucleus test (test method: EU B.12./OECD 474) in rats via the oral route;**

[2] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

c) **In case of positive result in the In vitro mammalian cell micronucleus test and if the chromosome centromere staining demonstrates both structural and numerical chromosomal changes, the Registrant shall perform a combined in vivo Mammalian erythrocyte micronucleus test (EU B.12./OECD 474) and an In vivo mammalian alkaline comet assay (OECD 489) in rat via oral gavage. The combined test has to be performed in accordance with OECD 489 and EU B.12./OECD 474 with an adequate treatment schedule for the combined assay (i.e. including a third dose administered on the 3rd day) as described by e.g. Bowen et al., 2011;**

d) **In case of clastogenic effect in the In vitro mammalian cell micronucleus test, an In vivo mammalian alkaline comet assay (OECD 489) in rats via oral gavage. DNA damage shall be assessed in forestomach, glandular stomach and liver;**

Tier 3:

In case of a positive result in the In vitro mammalian cell gene mutation test using the thymidine kinase gene[3]: the Transgenic rodent somatic and germ cell gene mutation assay (TGR, OECD 488) in mice or rat via inhalation, or via oral gavage. The test shall be conducted in mice or rat treated for 28 days. Tissues shall be harvested three days after the cessation of the treatment. Mutation frequency shall be assessed in nasal tissue, liver and bone marrow if inhalation is chosen, or in forestomach, glandular stomach, liver and bone marrow if oral route is chosen. Germ cells shall be sampled and stored. Cells shall be sampled from seminiferous tubules in addition to spermatozoa from the vas deferens/cauda epididymis. The germ cells shall be analysed for mutation frequency only in the case where positive test results are obtained for any of the somatic tissues;
OR
In vivo mammalian alkaline comet assay (Comet Assay, test method OECD 489) in rat via oral gavage. DNA damage shall be assessed in forestomach, glandular stomach and liver.
Points to be considered by the Registrant(s) in choosing which of the two above tests they should perform are given in section III.

If available, the Registrant(s) may use the information not reported, e.g. the positive controls data, to re-evaluate the reliability of the in vivo chromosomal aberration rat study (Engelhardt and Klimisch, 1983; as further described in section III.3) and may consider using the data in a weight of evidence approach before performing the in vitro micronucleus test and the following in vivo tests as requested in the above mutagenicity testing strategy.

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall also submit the following information regarding the registered substance subject to the present decision:

[3] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

4. Further information regarding derivation of dermal DNEL as specified in Section III

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA an update of the registrations(s) by exact date **23 October 2017**[4] containing the information required by this decision under point 1, 2, 4 and, 3 Tier 1, Tier 2a, Tier 2b, Tier 2c or Tier 2d depending on the results of the Tier 1 mutagenicity test, and an update of the Chemical Safety Report.

Pursuant to Article 46(2) of the REACH Regulation, and depending on the results of the Tier 2a mutagenicity test, the Registrant(s) shall submit to ECHA an update of the registration(s), containing the information required by this decision under point 3 Tier 3 and an update of the Chemical Safety Report:

- by **15 October 2018**[5] if the OECD 489 is conducted, or
- by **15 April 2019**[6] if the OECD 488 is conducted.

III. Statement of 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".

The following analysis presents the Registrant(s)'s justification for the proposed grouping approach and read-across hypothesis, together with the ECHA's analysis of the justification in both a generic and an endpoint-specific context.

a. Introduction of the grouping approach and read-across hypothesis proposed by the Registrant(s)

According to the Registrant(s), the substance subject to the present decision can be grouped with other substances for the purpose of the read-across in a category that is named "Acrylate Acid and Esters Category".

1. The Registrant(s) state that "The Acrylic Acid and Esters category is defined as a structurally related group of substances including acrylic acid (CAS No. 79-10-7), methyl acrylate (CAS No. 96-33-3), ethyl acrylate (CAS No. 140-88-5), n-butyl acrylate (CAS No. 141-32-2), i-butyl acrylate (CAS No. 106-63-8), t-butyl acrylate (CAS No. 1663-39-4), and ethyl hexyl acrylate (CAS No. 103-11-7). All of these chemicals have a common Structure-Activity-Relationship (SAR) to serve as the

[4] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

[5], [6] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

technical basis for the category",

2. The Registrant(s) further state that the category members consist of "molecules based on acrylic acid, and includes esters of the acid of increasing carbon chain length", and that the category is based on the following REACH criteria:
   [1] the sharing of a common functional group (all are esters of acrylic acid),
   [2] common chemical classes,
   [3] an incremental change in chain length across the category, and
   [4] common breakdown products for related acids and esters

3. According to the Registrant(s), because the category members have "been shown to be metabolized in the mammalian body in minutes to acrylic acid and the corresponding alcohol, they can be considered to constitute a chemical category", i.e. criteria 4 above. In addition, since the substances "exhibit similarity in their physicochemical properties and toxicological properties in mammals data gaps for mammalian toxicity can be addressed by read-across between category members".

   The Registrant(s) have used the grouping and read-across approach to predict the properties of the substance subject to this decision for the following endpoints: pre-natal developmental toxicity, two-generation reproductive toxicity and mutagenicity. It is noted that the Registrant(s) have also used weight of evidence approach for the adaptation.

b. Information submitted by the Registrant to support the grouping approach and read-across hypothesis

Concerning the grouping and read-across hypothesis, the Registrant(s) have provided a document of Justification for acrylate category including a data matrix indicating where available and adequate data on Acrylate Category Members exists (for Physicochemical properties, Environmental chemistry, Ecotoxicity, Human health effects), and a data matrix with data on physicochemical properties for the category members to indicate similarity; further information in their response to the draft decision on the reproductive toxicity of tert-butyl acrylate, ethyl acrylate, 2-ethyl hexyl acrylate and the metabolites acrylic acid and butanol; and information provided in the individual registration dossiers of the category members.

c. Analysis of the proposed grouping approach and read-across hypothesis in light of the requirements of Annex XI, 1.5.

Based on the information provided, ECHA understands that the category hypothesis is based on

1. Structural similarity and common Structure-Activity-Relationship (SAR) (*'common Structure-Activity-Relationship (SAR) to serve as the technical basis for the category'*). It is noted that the Registrant(s) have not explained what this SAR between the included acrylates is based on. In addition, the Registrants have provided structural formula for the category members but have not addressed the structural differences, such as branching and different chain length of the parent compounds, and the impact of these differences on the toxicokinetic and toxicological properties of the category members as explained further below.

2. ECHA considers that in comparison with the REACH definition of an acceptable category as set out in the REACH guidance document R.6 the proposed

acrylate category fulfils criteria *[1] the sharing of a common functional group,* in that all are esters of acrylic acid.

The proposed category also fulfils criteria *[2] common chemical classes*, in that the members are esters of acrylic acid.

The Criteria *[3] an incremental change in chain length across the category,* is not fulfilled because it is not only the carbon chain length that is increased across the category but also additional branching is introduced for several acrylate members (iso-butyl acrylate, tert-butyl acrylate, 2-ethylhexyl acrylate) that could potentially influence the toxicological properties of the substances.

The criteria *[4] common breakdown products for related acids and esters* is not fulfilled since there is no metabolism data for the branched acrylate members provided by the Registrant(s).

Substances used for the read-across approach by the Registrant(s) for assessing adverse effects on sexual function and fertility was methyl acrylate in the technical dossier; further data for read-across from tert-butyl acrylate was added in the Registrant(s) response to the draft decision. Substances used for the read-across approach by the Registrant(s) for assessing developmental toxicity was methyl acrylate in the technical dossier; further data for read-across from 2-ethyl hexyl acrylate and ethyl acrylate was added in the Registrant(s)' response to the draft decision. In addition, read-across to the metabolites acrylic acid and butanol was used in the assessment of adverse effects on fertility and developmental toxicity. Substances used for the read-across approach by the Registrant(s) for mutagenicity was methyl acrylate and ethyl acrylate in the technical dossier. The other category members or metabolites were not used in the read-across assessment, however, the Registrant(s) state that *"The chemical class and the metabolic degradation products give no suspicion that n-butyl acrylate has a genotoxic potential in vivo."*

Thus, both linear and branched category members are used in the read-across assessment of reproductive toxicity (both adverse effects on sexual function and fertility, and developmental toxicity), but the branched acrylate esters were not included in the assessment for mutagenicity. In the updated Category justification document, the Registrant(s) refer to recent in vitro metabolism studies conducted also with branched category members ([redacted]). However, the Registrant(s) have not provided results for all compounds and therefore the assumption about the metabolism of the non-linear acrylates being similar to the linear acrylates has not been demonstrated. Moreover, acrylic acid is stated to be the common metabolite of the majority of the esters of acrylic acid that are included as category members. However, no information to demonstrate that acrylic acid is formed for the branched category members was provided by the Registrant(s). Moreover, one in vitro metabolism study of tert-butyl acrylate demonstrated little or no hydrolysis by a porcine hepatic esterase.

The Registrant(s) have stated that *"the acrylate esters have been shown to be metabolized in the mammalian body in minutes to acrylic acid and the corresponding alcohol"*. However, the information on the rate and extent of hydrolysis of parent compounds of all category members is not sufficient for

the category to be considered acceptable as explained below. The rate should be demonstrated to be fast (within minutes) and complete so that the systemic exposure is (almost) entirely to the metabolites (and not the parent substance) for acceptance of the category justification.

Methyl, ethyl and butyl acrylate have been demonstrated to be hydrolysed to acrylic acid in rat liver, kidney and lung homogenates in vitro (Miller et al., 1981). In rat whole blood the t½ was 3.6, 4.6, and 7.1 minutes for disappearance of methyl, ethyl, and butyl acrylate, respectively (Miller et al., 1981). However it was unclear if hydrolysis of the parent compounds was responsible for the disappearance since this was not associated with an increase of acrylic acid.

Butyl acrylate was tested for relative rates of hydrolysis by porcine hepatic esterase in vitro. Conversion rates ranged between 54 - 69 µmol/min/mg protein (4 - 33 %) after 2 minutes and 43 - 72 µmol/min/mg protein (10 - 68 %) after 5 minutes of incubation at 37°C ([redacted]). Based on the data the hydrolysis is considered not complete. In addition, the metabolites were not identified. A preliminary (not peer-reviewed; low number of replicates and no statistics) in vitro study ([redacted]) indicated almost complete hydrolysis of butyl acrylate to acrylic acid within 5 minutes (disappearance of butyl acrylate with concomitant increase of acrylic acid) in S9 fraction from rat liver. However, this was not seen in whole blood or blood plasma. Although the hydrolysis seems to be rapid, the formation of butanol was not demonstrated. Butyl acrylate was also reported to undergo rapid carboxylesterase hydrolysis in vitro in nasal mucosa supernatant of mice, the apparent Km and Vmax values were 1.41 mM and 0.141 mM/min respectively. The specific activity of nasal carboxylesterase was found to be equivalent to that of the liver and greater than that of the kidney, lung or blood (Stott and McKenna, 1985). In vivo studies on the distribution and elimination of radiolabelled butyl acrylate after oral administration demonstrated that up to 75% of butyl acrylate was eliminated as CO_2 at 24 h and elimination in urine and feces accounted for approximately 10% and 2% of the administered dose. When radiolabelled butyl acrylate was administered via inhalation, elimination as CO_2 was up to 45% of the administered radiolabel. Elimination in urine and feces accounted for approximately 16 and 1% of the dose, respectively. It is stated by the Registrant(s) that these results show that the major portion of butyl acrylate was hydrolysed by carboxyesterase to acrylic acid and butanol and eliminated as CO_2. However the formation of the metabolites acrylic acid and butanol was not demonstrated. Moreover, the Registrant(s) stated that the elimination at 75% occurred at 24h which is not considered as rapid.

Methyl acrylate has been shown to undergo rapid carboxylesterase hydrolysis in vitro in the supernatant of the nasal mucosa of mice, the apparent Km and Vmax values were 3.14 mM and 0.241 mM/min respectively (Stott and McKenna 1985). In vitro studies of methyl acrylate in tissue homogenates demonstrated that the formation of acrylic acid was approximately 10 times greater in the liver compared to the lung (Miller et al., 1981). The amount of acrylic acid which formed in liver, kidney and lung homogenates was comparable to the amount of methyl acrylate which disappeared; hence, the rates of acrylic acid appearance and methyl acrylate disappearance were very similar during the total (20 min) incubation period.

Studies of ethyl acrylate in tissue homogenates demonstrated hydrolysis rates to be ~20 times higher in liver homogenates than in kidney or lung homogenates (Miller et al., 1981).

For the reasons explained above and due to brief reporting it is not possible to conclude if the hydrolysis of the linear category members is extensive and rapid, and therefore the impact of the parent compounds cannot be excluded. Therefore, the read-across from butyl, methyl and ethyl acrylate is not considered acceptable.

Isobutyl acrylate was shown to be rapidly hydrolyzed by mammalian esterase after 2 and 5 minutes (at 3 - 27 % and 7 – 57% respectively) of incubation at 37°C.

As stated above, little or no hydrolysis of tert-butyl acrylate in vitro by porcine hepatic esterase was evident.

For 2-ethyl hexyl acrylate there were no data supporting rapid hydrolysis, or that acrylic acid is formed.

For the reasons explained above and due to brief reporting it is not possible to conclude that the hydrolysis of the non-linear category members is extensive and rapid, and therefore the impact of the parent compounds cannot be excluded. In addition, the metabolites were not identified. Therefore, the read-across from isobutyl acrylate, tert-butyl acrylate and 2-ethyl hexyl acrylate is not considered acceptable.

3. It is further noted that the Registrant(s) state that "*in the absence of measured data, toxicity of category members can be estimated based on toxicity data derived from testing acrylic acid and, to a lesser extent, the alcohols associated with the esters.*" As stated above, the alcohols formed (if they are formed) are structurally different and consequently, their toxicological profiles may be different. Therefore, the prediction should be based both on the common metabolite acrylic acid and non-common alcohol metabolites.

   Due to linear and branched carbon chains with different lengths of the parent substances, the alcohols formed (if formed) are structurally different as stated above. No information on the formation of the corresponding alcohol metabolites of the category members and the subsequent metabolism of these has been provided. Information on the toxicity of these has not been provided by the Registrant(s). The Registrant(s) have not addressed these structural differences and their impact on the toxicokinetics and toxicological profile of the category members. In the absence of additional supporting information on the toxicity of the alcohol metabolites (except for n-butanol) it is concluded that the lack of this information impacts the possibility to predict.

d. Conclusion on the grouping and read-across approach

It is concluded that according to Annex XI, 1.5., the grouping and read-across approach for the Acrylate Acid and Esters Category is not adequately and reliably documented as the analysis of the structural differences of parent compounds and corresponding alcohol metabolites and their impact on the toxicokinetic properties

and (eco)toxicological profile is missing. In addition, the rate and extent of hydrolysis of category members has not been adequately addressed.

The information, data and reasoning provided by the Registrant(s) does not allow accepting the prediction from the source substances in the proposed acrylate category. It is considered that it is not possible to predict the properties in question for butyl acrylate by the available data of the proposed acrylate category members, together with the available data of the metabolites acrylic acid and butanol.

Consequently, it is concluded that the proposed read-across approach does not fulfil the requirements defined in Annex XI, 1.5. of the conditions for accepting read-across 'Being adequate for the purpose of classification and labelling and/or risk assessment.' As a result and based on the information analysed in the light of above deficiencies and based on the information provided, these substances cannot be used for read-across purposes for reproductive toxicity and mutagenicity as proposed by the Registrant(s).

The read-across approach is considered separately for each endpoint in which this approach has been applied.

1. **Extended one-generation reproductive toxicity study in rats, via the oral route (test method: B.56./OECD 443) without the Cohorts 2 and 3 and without the extension of Cohort 1B to mate the F1 animals to produce the F2 generation.**

The premating exposure shall be 10 weeks and the highest dose should be selected with the aim to induce some toxicity.

Initial concern
The available information did not enable eMSCA to conclude on the potential of the substance to cause adverse effects on sexual function and fertility as defined in Regulation (EC) No 1272/2008. The available information in the current registration does not give adequate basis to read-across to data on adverse effects on sexual function and fertility of the structural analogue methyl acrylate.

Moreover, the available information did not enable eMSCA to conclude on the potential of the substance to cause adverse effects on the development of the offspring. There is a data gap concerning whether the observed developmental toxicity is an indirect effect due to maternal toxicity or whether the developmental toxicity is a direct effect of the substance. In conclusion, available information in the current registration does not give adequate basis to read-across to data on adverse effects on the development of the offspring of the structural analogue methyl acrylate.

Summary of justification
It is considered that in this specific case, OECD 443 without extension of Cohort 1B and without Cohorts 2 and 3, adequately addresses the concerns expressed; potential reproductive effects for workers. Concerns related to adverse effects on sexual function and fertility for workers should be adequately covered without the extension of Cohort 1B; there seem to be no significant exposure for consumers or professionals, which is one element of the conditions triggering the extension of cohort 1B in the revised REACH Annexes IX and X, 8.7.3, column 2. The concern related to developmental toxicity (increased number of resorptions, decreased number of fetuses) is also expected to be addressed without information on the extension of Cohort 1B. Information on sexual function, fertility and developmental toxicity is needed to address the potential CMR properties and to provide

information to derive endpoint specific DNELs. For this purpose, to adequately address concerns on adverse effects on sexual function and fertility and on developmental toxicity, a 10-week premating and highest dose level showing some systemic or reproductive toxicity is needed.

Relevant available data - Adverse effects on sexual function and fertility
At present, there are no studies of butyl acrylate which address adverse effects on sexual function and fertility. An inhalation repeated dose toxicity studies (90-day in rat) did not indicate effects on reproductive organs (no effects were found in the seminal vesicles, prostate, epididymis, uterus, testes or ovary upon microscopic examination, and no effect on testis weight) in doses up to 546 ppm. A two years (6 hr/day, 5 days/week, for 24 months) combined chronic toxicity /carcinogenicity study with doses up to 135 ppm reported that organ weights were generally unaffected by treatment. Moreover, two oral repeated dose toxicity studies (90-days, rat) showed minimal indications of toxicity and no influence on reproductive organ weights at the doses tested (up to 84/111 (male/female) mg/kg bw/day and 150 mg/kg bw/day respectively). However none of these studies can conclude on the integrity and performance of the male and female reproductive systems, including gonadal function, the oestrus cycle, mating behaviour, conception, gestation, parturition and lactation.

Evaluation of the read-across data and weight of evidence for fertility endpoint

In the technical dossier the Registrant(s) provided information with which they sought to fulfil the standard information requirement in Annex IX, 8.7.3.

The Registrant(s) are using grouping and read-across approach and weight of evidence approach for this endpoint and have provided data on n-butyl acrylate ([redacted]; Reininghaus et al. 1991; [redacted] Gorzinski SJ et al., 1982) its metabolites acrylic acid ([redacted]; Hellwig et al., 1997; [redacted] DePass et al., 1983) and butan-1-ol (butyl acetate as test substance [redacted]), and category members methyl acrylate ([redacted]) and tert-butyl acrylate ([redacted]).

Butyl acrylate
As summarized above, there are no studies of butyl acrylate which address adverse effects on sexual function and fertility. The Registrant(s) have included data from repeated dose toxicity studies and a chronic study as supporting evidence in the weight of evidence assessment for the reproductive toxicity of butyl acrylate. No effects on reproduction organs (organ weights or histopathology) were observed in the subchronic and chronic studies with butyl acrylate itself.

Acrylic acid
Rats were exposed via drinking water at doses 0, 500, 2500 and 5000 ppm (corresponding to approx. 0, 53, 240 and 460 mg/kg bw/day) in a two-generation reproduction toxicity study (Hellwig et al., 1997). The study indicated no effects on sexual function and fertility of the F0 or the F1 generation. Body weights were significantly reduced in F1 males (87% as compared to controls) at 5000 ppm (460 mg/kg bw) throughout the study. Body weight gains of the males, however, were generally similar to the respective control values (about 5% lower). Body weights in F1 females were significantly reduced during pre-mating (89% as compared to control) and were still statistically significantly lower during gestation and

[7] Full reference not available.
[8] Full reference not available.
[9] ECHA dissemination website

lactation at 5000 ppm. F1 and F2 pup body weights were statistically significantly lower at weaning (approx. 65-69% as compared to controls, $p<0.01$) at 5000 ppm and eye opening in F2 pups was statistically significantly delayed with 86.5 % of pups reaching criteria/litter compared to 93.2% in control (historical control range: 85-100%).

In a one-generation reproductive toxicity study in rat acrylic acid was tested at 0, 83, 250, 750 mg/kg bw/day in drinking water (DePass et al., 1983). Possible effects at high dose on gestation index and the number of live pups (not statistically significant; note that fertility index and litter size were unrepresentatively low in control) were reported. Body weight gains were markedly depressed for both sexes at the high dosage level (body weight gains were 69% of control in females, and 70% of control in males) and were statistically significant in these groups throughout the study. However, the final maternal body weight was 95% as compared to control.

In summary, results from one- and two-generation reproductive toxicity studies of acrylic acid indicates that there is concern for female fertility, pup viability and growth.

Butyl-acetate
A brief statement on a two-generation reproductive toxicity study of butyl acetate was included by the Registrant(s) to cover data of a reproductive toxicity study of butanol. The Registrants states: *'The assessment of the metabolic cleavage product n-butanol (tested as n-butyl acetate in a 2- generation study, gives also no suspicion of a fertility impairing potential.'* No justification to this read-across was provided, however, the metabolism of acetate to alcohol could be considered as "well-known" pathway as publicly available assessments are available (e.g. WHO, 2005). Furthermore, no data on this study was provided by the Registrant(s). At ECHA's dissemination site of registered substances the following information on this study was retrieved: Rats were exposed to butyl acetate at 0, 750, 1500, 2000 ppm, and 7 days per week (0, 1125, 2250, 3000 mg/kg bw/day) via inhalation. At 2000 ppm (3000 mg/kg bw/day) F0 female body weights were statistically significantly lower during pre-mating and during lactation. F1 maternal weights were statistically significant lower (13.1 to 16.9%) during gestation and throughout lactation. Histological lesions were observed in F0 and F1 males and females. At 2000 ppm F1 pup body weights were statistically significantly lower at PND1. F2 pup body weights were significantly lower at PND 7-21. Delays in attainment of post weaning developmental landmarks in F2 (auditory canal opening, eye opening) were reported.

In summary, no adverse effects on sexual function or fertility were reported for butyl acetate in this study. At maternal toxic doses pup body weights were significantly lower in F1 and F2. In addition, delays in post weaning development were indicated in F2 pups.

Methyl acrylate
The requirement of a reproductive toxicity test according to Annex X, 8.7.3 for butyl acrylate was adapted in the technical dossier with read-across to data of the structural analogue methyl acrylate. Methyl acrylate was tested in a two-generation reproductive toxicity study in rats via inhalation at 0, 5, 25, and 75 ppm ([redacted]). At the highest dose tested (75 ppm = 0.269 mg/l) the F0 and F1 gestation body weights were statistically significantly decreased at GD 21 (7% and 11% respectively). Dose-related histopathological effects (degeneration with regeneration) in the olfactory epithelium were reported in F0 and F1 males and females at all doses tested. There were no effects of treatment on the number of pups born live, number of pups born dead, or on litter size at any time interval in any exposure group for either generation. The final body weights of F1 weanling males and females from the 75 ppm group were approximately 6% lower than controls, not statistically significant. There were no treatment-related gross pathologic observations in F1 weanlings at any exposure level. In F2 weanlings, 2/81 males and 3/78

females from the 75 ppm exposure group had necrosis of the tail. All other gross pathologic observations from F1 and F2 weanlings were considered to be spontaneous alterations, unassociated with exposure to methyl acrylate.

In summary, there does not seem to be a concern for adverse effects on fertility or sexual function or developmental toxicity for methyl acrylate in this study.

Tert-butyl acrylate
A study according to OECD 422 of tert-butyl acrylate via inhalation in rat at 20, 60, 180 ppm ([redacted]) was identified by the Registrant(s) as a supporting study in the read-across to the acrylates category in their follow up response to the draft decision. Maternal toxicity in this study was manifested at the highest dose tested as slight irritation of the eyes and upper respiratory tract, reduced maternal body weight gain GD 0-20 and mortality on GD 18 and GD 20 (20%). Live birth index was statistically significantly decreased and the percentage of dead pups and pups cannibalized were statistically significantly increased. Pups body weights were statistically significantly decreased at day 1 and 4 postpartum. The Registrant(s) have assigned reliability 1 to this study, however, considering the excess toxicity at high dose, the dose levels are not considered appropriate for reliable interpretation of results.

In summary, the reported results indicate developmental toxicity as decreased pup viability and pup body weight, however the interpretation of the results are considered to be compromised by the excessive maternal toxicity.

The Registrant(s)' conclusion on the fertility endpoint
Overall, the provided data has been concluded by the Registrant(s) in the following statement: *The overall weight of evidence based on the data on n-butyl acrylate itself (reproduction organs), 2-generation studies with acrylic acid, methyl acrylate, an extended OECD 422 with tert-butyl acrylate and a 2-generation study with n-butyl acetate gives no indication that n-butyl acrylate might have an reproduction toxic effect.*

Conclusion on the fertility endpoint
The Registrant(s) have sought to adapt this information requirement using a read-across and weight of evidence approach, and have provided studies conducted with the registered substance, its metabolites and category members.

The Registrant(s) claim that due to structural similarity and rapid hydrolysis of the category members, toxicity of butyl acrylate could be derived from testing acrylic acid and alcohols associated with the esters. However, as stated in section III.0. above, the analysis of the structural differences of parent compounds and the corresponding metabolites and their impact on the properties and (eco)toxicological profile of the category members is missing. In addition, sufficient information on the rate and extent of hydrolysis for the parent compound has not been provided. Therefore, the adaptation of the information requirement suggested by the Registrant(s) cannot be accepted and the possibility to predict from the other category members and from metabolites of butyl acrylate to the target substance as required in Annex XI, 1.5. has not been demonstrated.

In addition, according to Annex XI, 1.2., '*There may be sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion.*' When sufficient weight of evidence is available and adequate and reliable documentation have been provided, further testing to meet the information requirements may be omitted. The Registrant(s) have not

provided sufficient evidence to demonstrate that the potential reproductive toxicity of butyl acrylate can be concluded from the available data.

Moreover, it is considered that the inclusion of read across data from the structural analogue methyl acrylate to aid in the assessment of adverse effects on fertility and developmental toxicity is not an appropriate approach in the case of butyl acrylate for the following reasons. In a comparative study of acrylates by Saillenfait et al (1999) both methyl acrylate and butyl acrylate were studied and a greater (approximately 3-4-fold) potency in the form of maternal toxicity was demonstrated for methyl acrylate than for butyl acrylate. Furthermore, in comparative toxicity studies where a number of acrylates were ranked according to their toxicity the following order was identified: methyl acrylate > ethyl acrylate > butyl acrylate, indicating decreased toxic activity of acrylic esters with increasing molecular weight (Autian, 1975). The higher irritancy (in olfactory epithelium and in the corneal parenchyma) of methyl acrylate as compared with butyl acrylate has been speculated to be partly because of the differences in diffusibility and water/lipid solubility (Tanii and Hashimoto, 1982). Other studies point to the contribution of the epithelial metabolism on the uptake and effects of irritants (Stott and McKenna, 1984). It is considered that methyl acrylate induces severe local effects via inhalation and it is therefore not feasible to achieve higher doses in animals to study systemic effects including reproductive toxicity. Butyl acrylate is less irritating at equimolar concentrations compared to methyl acrylate and may be administered at higher doses. Systemic effects, including reproductive toxicity, suspected to occur at higher doses than at dose levels where local effects are observed may therefore not be suitable for read across between methyl acrylate and butyl acrylate since dose range selection in developmental toxicity studies of methyl acrylate may not reflect the effect levels of the potential developmental toxicity of butyl acrylate. In addition, considering the oral route as an alternative exposure route to circumvent the difference in potency of the local irritative effect in the respiratory tract there is one study indicating that methyl acrylate and ethyl acrylate at equimolar doses as butyl acrylate and acrylic acid gave profound gastric toxicity when administered via oral gavage in corn oil at 2 mmol/kg in male F344 rats (Ghanayem et al., 1985). In contrast, no gastric toxicity was observed after administration of butyl acrylate or acrylic acid. This puts additional weight on the consideration of the applicability of read-across between butyl acrylate and methyl acrylate as limited.

Overall, when evaluating the validity of read-across to acrylate structural analogues for reproductive toxicity data it may also be taken into account that there is no well-established convincing database of studies of reproductive toxicity for acrylates, making this endpoint not suitable for a chemical category approach.

Therefore, it is considered that the available information does not provide the information required by Annex X, Section 8.7.3. The conditions set out in Annex XI, Sections 1.2 and 1.5 for adaptions of information requirements are not fulfilled mainly with regards to 'being adequate for the purpose of classification and labelling and/or risk assessment.' Consequently there is an information gap and it is necessary to provide information for this endpoint.

Relevant available data - Adverse effects on the development of the offspring
The present data are equivocal and not without a concern for adverse effects on the development of the offspring. The data provided by the Registrant(s) for adverse effects on the development of the offspring is discussed in section III.2.

Evaluation of the read-across data and weight of evidence for developmental endpoint
In the technical dossier and in their comments to the draft decision the Registrant(s) provided information with which they sought to fulfil the standard information requirement

in Annex X, 8.7.2. and 8.7.3.

The Registrant(s) are using grouping and read-across approach and weight of evidence approach for this endpoint and have provided data on n-butyl acrylate (Merkle and Klimisch, 1983; [redacted]; Saillenfait et al., 1999), its metabolites acrylic acid ([redacted]; Hellwig et al., 1997; [redacted][10]; DePass et al., 1983; [redacted][11]; Neeper-Bradley et al., 1997; [redacted][12]; Klimisch and Hellwig, 1991;) and butan-1-ol (butyl acetate as test substance in study report 2007, 2010[13], Ema et al., 2005, Nelson et al., 1989; Sitarek et al., 1994), and category members methyl acrylate ([redacted]; Saillenfait et al., 1999), ethyl acrylate (IATG, 1982; Saillenfait et al., 1999), 2-ethyl hexyl acrylate (Saillenfait et al., 1999) and tert-butyl acrylate ([redacted]).

Summaries of studies provided are included in the section above (*Evaluation of the read-across data for fertility endpoint)* and in section III.2.

Specification of the test

In substance evaluation, the study design of OECD 443 should reflect the concern and this is also an approach in the Annexes IX and X, 8.7.3 of REACH, where extension of Cohort 1B and the need to include Cohorts 2A/2B and 3 are triggered based on substance specific reasons. Testing according to OECD 443 is requested here, excluding extension of Cohort 1B, and excluding the DNT/DIT cohorts (Cohorts 2A/2B and Cohort 3), and with the stated specifications, for the following reasons:

Inclusion/exclusion of the extension of Cohort 1B
Butyl acrylate is registered at the highest tonnage band but the exposure is mainly limited to workers and it is not expected to lead to exposures of consumers and professional users. Consequently, butyl acrylate does not fulfil the exposure based criteria indicated in the revised Annexes IX and X, 8.7.3 of REACH for triggering of the extension of Cohort 1B to mate the F1 animals to produce the F2 generation.

Inclusion/exclusion of Cohort 2A/2B
No particular concern for (developmental) neurotoxicity was identified for Cohorts 2A and 2B, and thus these cohorts should be excluded.

Inclusion/exclusion of Cohort 3
No particular concern for (developmental) immunotoxicity was identified for Cohort 3 and, thus, it should be excluded.

Duration of the premating exposure
The premating exposure period for the P animals shall be extended to 10 weeks to cover the complete spermatogenesis and folliculogenesis before mating and to allow to evaluate fully the potential effects on reproductive performance in P animals and effects in their offspring (F1).

Selection of exposure route
According to the test method OECD 443, if the exposure route deviates from the recommended oral route, a justification is required. In the case of butyl acrylate, inhalation is the relevant human exposure (and dermal exposure to some extent).

[10] Full reference not available.
[11] Full reference not available.
[12] Full reference not available.
[13] ECHA dissemination website

Repeated inhalation of butyl acrylate causes irritating effects to nasal and respiratory mucosa and the eyes at dose levels starting from 100 ppm. Local irritation may cause stress and reduced body weight in dams and may not allow proper evaluation of the relationship between real systemic effects in dams with developmental toxicity. In case a substance causes respiratory irritation, an oral route would be more appropriate. This may allow higher systemic dose levels relevant for classification and labelling purposes to be used. The Registrant(s) are therefore requested to perform an EOGRTS via the oral route.

Selection of the dose levels
The study shall include at least three treatment dose levels and a concurrent control. The highest dose should be selected with the aim to induce some toxicity.

Conclusion
An extended one-generation reproductive toxicity study of butyl acrylate at relevant dose levels is foreseen to generate data both on adverse effects on sexual function and fertility and developmental toxicity. New data may allow the Registrant(s) to establish endpoint-specific NOAELs that were not available in the current registration and it is expected that the data derived from the test will aid in the evaluation of developmental toxicity and potentially arriving at a conclusion regarding classification according to criteria in Annex I to CLP (Regulation (EC) No 1272/2008).

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision: Extended one-generation reproductive toxicity study in rats, via the oral route (test method: EU B.56./OECD 443) without Cohorts 2 and 3 and excluding the extension of Cohort 1B to mate the F1 animals to produce the F2 generation.

The Registrant(s) objected the proposed test requirement and disagreed with certain methodological options. The following reasoning was provided by the Registrant(s):" i) Currently no laboratory has any experience in performing an inhalation EOGRTS and especially not with cohorts, and only very few (if any at all) will have the technical equipment, which results in extreme uncertainties and difficulties. ii) The proposed test seems technically not feasible, e.g. within the F1 generation 760 animals will have to be exposed in parallel 6 hrs/day, which already takes within handling time 8 hours. Afterwards all further tests (i.e. behavioural tests) will have to be performed; there is no experience/ validation in performing these tests at night time. iii) Already now the capacity for EOGRTS via oral route is very limited (the oral route is not applicable for n-butyl acrylate) and identifying an appropriate laboratory and establishing the method for inhalation will take years, therefore no results can be realistically expected within at least the next 5 years. iv) the only feasible study to be performed will be a two-generation reproduction toxicity study with butyl acrylate according to OECD 416 using inhalation as the most appropriate route of exposure under GLP conditions. With this study, also a further teratogenicity study will not be needed as all possible uncertainties concerning reproduction and developmental toxicity will already be addressed in this two-generation study via inhalation route."

The arguments provided by the Registrant(s) are not an incentive to remove the request of an EOGRTS. In Annex IX 8.7.3 and Annex X 8.7.3 of the REACH regulation the requirement of a two-generation reproductive toxicity test (OECD 416) has been replaced by an EOGRTS (EU B.56./OECD 443 as of 13 March 2015). The draft decision is now amended to request the Registrant to perform an EOGRTS via the oral route. Considering the Registrants comments, this will enhance the feasibility of the method. In addition, Cohorts 2 and 3 have been removed from the information request further reducing the workload.

2. Prenatal developmental toxicity study in rabbits, via the oral route (test method: EU B.31./OECD 414)

Concern
The available information did not enable the evaluating MSCA to conclude on the potential of the substance to cause adverse effects on the development of the offspring. The available data is equivocal and there is no adequate basis for read-across to data on adverse effects on the development of the offspring of the structural analogue methyl acrylate. There is concern related to the lack of information from a second species developmental toxicity study that is a standard information requirement in REACH Annex X.

Summary of justification
At present there is not adequate information to conclude about potential risks related to pre-natal developmental toxicity of the substance and to ensure safe use via implementation of adequate risk management measures including hazard classification. Therefore the request of a pre-natal developmental toxicity study in a second species is deemed justified and proportionate to clarify the concern expressed: potential reproductive effects for workers. Based on available data, the concern for developmental toxicity is mainly on increase in resorptions and reduction in fetal weight. A prenatal developmental study is expected to detect those findings with better accuracy than an EOGRTS (information request 1 in this decision). ECHA also considers that classification and labelling for developmental toxicity, including categorisation, would be more adequately addressed in OECD 414 considering the concern identified.

Relevant available data
The present data are equivocal and not sufficient to exclude the concern for adverse effects on the development of the offspring. Moreover, according to the Guidance on the Application of CLP Criteria (Version 4.0, 2013) developmental effects which occur even in the presence maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity.
Three pre-natal developmental toxicity studies for butyl acrylate are available in the present registration but the available data are not conclusive.

Merkle and Klimisch (1983) reported an increased percentage of resorptions and a reduced number of fetuses in rats exposed to butyl acrylate (doses 0, 25, 135, 250 ppm) via inhalation on GD 6-15. The Registrant(s) argue in the CSR that the observed developmental toxicity is explained by maternal toxicity manifested as decreased gestational body weight gain at 135 ppm and 250 ppm (16% less than control, $p<0.05$ and 31% less than control, $p<0.01$ respectively). During the exposure, 135 ppm led to distinct discharge from the eyes and noses and to ruffled fur. After inhalation of 250 ppm these symptoms were even more pronounced. The observed local irritation may cause stress and affect body weight in the dams. It is noted, however, that the absolute maternal weight at 135 ppm and 250 ppm at GD20 is only slightly reduced (5% and 10%, $p<0.01$, respectively) compared to control and that the reduced weight gain in exposed dams (135 ppm) compared to control dams approximately corresponds to the total weight of the reduced number of fetuses (approx. 3 fetuses of 4 g → 12 g). Therefore, the maternal toxicity (reduced weight gain) cannot explain the findings of developmental toxicity and the conclusion from the study indicates that butyl acrylate may induce developmental toxicity (resorptions).

In a second pre-natal developmental study in rats exposed to butyl acrylate (doses 0, 100, 200, 300 ppm) via inhalation on GD 6-20 (Saillenfait et al., 1999) reduced fetal weight (7% less than control, $p<0.05$ and 26% less than control, $p<0.01$ at 200 and 300 ppm respectively) in combination with significant reduced absolute gestational maternal weight

gain at 100 ppm (18 g versus 32 g, $p<0.05$) or significant weight loss at 200 ppm and 300 ppm (-16 g, $p<0.01$ and -60 g, $p<0.01$ respectively). The maternal gestational body weight at GD 21 was 96, 88, and 71% of control at 100, 200 and 300 ppm respectively. No significant effects on number of live fetuses or litters, percentage of resorption sites per litter, developmental effects or malformations were observed. There seems to be no clear developmental toxicity based on this second study, however, the fetal body weight was slightly reduced and concern based on the increased resorptions observed in the first study remains unsolved.

In a third pre-natal developmental toxicity study butyl acrylate (doses 100, 1000, 1500, 2000, 2500, 3000, 4000 mg/kg bw) was administered to mouse via oral gavage on GD 6-15. At doses ≥1000 mg/kg bw mortality was 3.3-6.7% and effects on maternal weight was significant at doses ≥1500 mg/kg bw ([redacted]). Fetal body weights were also reduced from 1500 mg/kg bw. At 2500 and 3000 mg/kg bw the percentage of resorptions was significantly increased. In these dose groups, the number of fetuses with malformations was also significantly increased. The reliability of the study is not assignable since the full study report is not available to the Registrant(s) or the eMSCA. Therefore the validity of the results is questionable. Moreover, mortality was reported at each dose level at or above the limit dose (1000 mg/kg bw/day) which is considered as excessive toxicity. The lowest dose level in the study (100 mg/kg bw/day) was the only dose level without mortality. Therefore, the selected dose spacing is considered to be inappropriate.

To conclude, based on the available data on butyl acrylate, there is a concern for developmental toxicity in form of increased amount of resorptions in one rat study (Merkle and Klimisch, 1983) which was not solved in another rat study (Saillenfait et al., 1999) or the mouse study ([redacted]). ECHA considers existing data not sufficient to clarify the concern and ensure that risk is under control.

In addition, PNDT studies performed on two species are required according to Annex X, 8.7.2 and rat and rabbits are the preferred rodent and non-rodent species according to test guideline EU B.31./OECD 414. In the present case, no PNDT study on rabbits is present. ECHA considers that there is a data gap for a developmental toxicity test on the rabbit as a second species.

Evaluation of read-across data and weight of evidence for developmental toxicity endpoint
In the technical dossier and in their comments to the draft decision the Registrants provided information with which they sought to fulfil the standard information requirement in Annex X, 8.7.2.

The Registrant(s) are using grouping and read-across approach and weight of evidence approach for this endpoint and have provided data on n-butyl acrylate (Merkle and Klimisch, 1983; Rohm and Haas Co, 1979; Saillenfait et al., 1999), its metabolites acrylic acid ([redacted][14]; Neeper-Bradley et al., 1997; [redacted][15]; Klimisch and Hellwig, 1991;) and n-butanol (Ema et al., 2005, Nelson et al., 1989; Sitarek et al., 1994), and category members methyl acrylate ([redacted]; Saillenfait et al., 1999), ethyl acrylate (IATG, 1982; Saillenfait et al., 1999), and 2-ethyl hexyl acrylate (Saillenfait et al., 1999).

Butyl acrylate
Results from prenatal developmental toxicity studies on butyl acrylate itself are described in the section above (*Relevant available data*).

[14] Full reference not available.
[15] Full reference not available.

Acrylic Acid

Rats were exposed to acrylic acid at 0, 40, 120, and 360 ppm (corresponding to approx. 0, 0.12, 0.36 and 1.08 mg/L) during GD 6 to 15 (sacrifice at GD 20) in a developmental study according to OECD 414 ([redacted][16]; Klimisch and Hellwig, 1991;). In the dams, irritation of the respiratory tract and the eyes was observed in the highest dose group. At the highest dose tested (360 ppm) there was a slight but statistically significant decrease (92% less as compared to control) in body weight at GD 20. There were no signs of group-related trends or significant differences between groups in terms of pre-implantation losses, live fetuses, or resorptions. There were also no signs of group-related differences in the incidences of abnormalities, variations, or retardations in the fetuses in terms of general appearance, fetal body weights and the conditions of the internal organs or the skeleton.

Results from the study by Saillenfait et al (1999) were not presented for the read-across or weight of evidence approach by the Registrant(s). However, as stated above, this study compared the developmental toxicity of various acrylates (acrylic acid, methyl acrylate, ethyl acrylate, 2-hydroxyethyl acrylate, hydroxypropyl acrylate) and was included for end point data on butyl acrylate, ethyl acrylate, methyl acrylate and 2-ethyl hexyl acrylate. Acrylic acid was tested in rats at 0, 50, 100, 200, 300 ppm via inhalation at GD 6-20. The body weight gain of the dams was significantly reduced at 200 and 300 ppm and the body weight at GD 20 was decreased to 85% as compared to control at 300 ppm. No treatment-related effects were reported in terms of numbers of implantation sites, live fetuses, non-live implants or resorptions. Fetal body weight was dose-dependently reduced and statistically significant at 300 ppm (9% lower than control).

Groups of 16 pregnant rabbits were exposed to acrylic acid at 0, 25, 75, and 225 ppm (corresponding to approx. 0.075, 0.224, 0.673 mg/L) during GD 6 to 18 (sacrifice on GD 29) ([redacted][17]; Neeper-Bradley et al., 1997). Dose-related clinical signs (as perinasal/perioral wetness and nasal congestion, as well as reduced body weight gain and food consumption) were observed in the 75 and 225 ppm groups. The overall pregnancy rate was equivalent for all groups (94-100 %). There were no effects on the number of ovarian corpora lutea, the number of total viable or non-viable (early and late resorptions and dead fetuses) implantations/litter. Percentage live fetuses and sex ratio were equivalent across groups. Fetal body weights were unaffected by test substance exposure. There were no exposure-related increases in the incidences of external, visceral or skeletal malformations or variations.

Results from one- and two-generation reproductive toxicity studies of acrylic acid (DePass et al., 1983; Hellwig et al., 1997) were included by the Registrant(s) to contribute to the assessment of adverse effects on fertility and sexual function, however, these studies should also be included in the total weight of evidence for developmental toxicity. The results of these studies are briefly summarized in section III. 1, indicating effects on pup viability and reduced pup growth and delayed eye opening.

In summary, inhalation exposure of pregnant rats and rabbits to atmospheres containing acrylic acid at concentrations up to 360 ppm (rats) and 225 ppm (rabbits)

[16] Full reference not available.
[17] Full reference not available.

produced no significant evidence of developmental toxicity in either species. The three studies of developmental toxicity of acrylic acid can be considered to be reliable, however, the highest dose selected for the Klimisch and Hellwig (1991) study in rat and the Neeper-Bradley et al (1997) study in rabbit may not be sufficiently high for the purpose of investigating reproductive toxicity.

n-Butanol

In a study by Nelson et al. (1989a), groups of 15-18 female Sprague-Dawley rats were exposed at 8000, 6000, 3500, or 0 ppm (24.7, 18.5 or 10.8 mg/L) 1-butanol via inhalation on GD 1-19 (dams were sacrificed on GD 20). 8000 ppm produced narcosis in approximately one-half of the dams. Two of eighteen dams at 8000 ppm died during the exposure period. Food consumption was decreased in the 6000 and 8000 ppm exposed dams. Fetal weights were slightly decreased at 6000 and 8000 ppm groups. External fetal malformations were not observed. There were no differences in malformation rates (skeletal or visceral) or in rates of commonly observed variations. However, there was a slight increase in the percent of fetuses with any skeletal variation or malformation (mainly rudimentary cervical ribs) in the 8000 ppm group but not in the lower two exposure groups.

In a behavioral teratogenicity study (Nelson et al. 1989b), there were no behavioral teratogenic effects found in rats in doses up to 6000 ppm (18.5 mg/L) and no general maternal or paternal toxicity was reported at the same doses.

In the study of Sitarek et al. (1994), 0, 0.24, 0.8 and 4% n-butanol (0.3; 1.0 and 5.0 g/kg/day) was administered to rats in drinking water 8 weeks premating, during mating (max. 3 weeks) and gestation day 0–20. No toxicity was reported for the parental animals exposed to butanol and the general appearance and behavior as well as body weight gain, food and liquid intake of the exposed animals were similar to that of the control animals.

The unit of statistical analysis for developmental toxicity in this study was the individual fetus, not the litter. Developmental effects were reported at the highest dose tested and in 2 fetuses in the low dose group. At 5000 mg/kg bw the crown-rump length was decreased (mean of 4.0 to 3.8 cm for the control and treated group, respectively). There was a slight increase in the percent of fetuses with any skeletal variation or malformation (mainly rudimentary cervical ribs) in the 5000 mg/kg bw group and in the low dose group. These effects were not reported in the control fetuses. CNS defects were reported as dilation of either the subarachnoid space or lateral and/or third ventricles of the brain, or external or internal hydrocephalus. Two of the 61 control fetuses examined for visceral anomalies had dilatation of the lateral and/or third ventricles of the brain, while none had dilatation of the subarachnoid space or external or internal hydrocephalus. Dilated renal pelvis was also observed. According to the Registrant(s) the results of this this study should not be regarded as selective fetal effects.

In a prenatal developmental toxicity study (not performed according to OECD TG), pregnant rats were given drinking water containing 1-butanol at 0.2%, 1.0% or 5.0% (corresponding to ca. 316, 1454 or 5654 mg/kg/day) on GD 0 to 20 (sacrificed on GD 20) (Ema et al. 2005). A significant decrease in maternal body weight gain accompanied by reduced food and water consumption was found at 5.0%. However, no data on body weights of the dams were reported. No significant increase in the incidence of pre- and postimplantation embryonic loss was observed in any groups treated with 1-butanol. The body weights of male and female fetuses were significantly lower in the 5.0% group than in the control group. The total number of

fetuses with skeletal variations was significantly increased at 5.0% (69 (20) vs 28 (11), $p<0.01$), but the number of fetuses with individual skeletal variations was not significantly increased, except for fetuses with short supernumerary ribs at 5.0%. A statistically significantly lower number of forepaw proximal phalanges was observed at 5.0%. Membranous ventricular septum defect occurred in one fetus of the control and 0.2% groups and 3 fetuses in 3 dams of the 5.0% group.

The conclusion of the Registrant(s) on developmental toxicity of butanol was:
'Results from valid experimental studies showed no indication, that Butan-1-ol caused fetotoxic or teratogenic effects in doses below maternal toxic doses in rats. Results from an inhalation study in rabbits with the analogous Butyl acetate, CAS No. 123-86-4, support this assessment in a second species.'

The PNDT study in rabbit of butyl acetate mentioned by the Registrant(s) is not described any further in their comments. ECHA considers the results of this study to be of limited value since only one dose (7260 mg/m^3) was tested. Atmospheres of butyl acetate at about 10000 ml/m^3 are more or less saturated, meaning that the limit dose for inhalation of butyl acetate was not reached in this case. The developmental effects reported at the only dose tested were increased incidence of minor developmental effects.

Results from a two-generation reproductive toxicity study of butyl acetate (Study report 2007, 2010)[18] was included by the Registrant(s) to contribute to the assessment of adverse effects on fertility and sexual function of butanol (see section III.1 for further details on the justification to include this study), however, this study should also be included in the total weight of evidence for developmental toxicity. The results of the study are briefly summarized in the section III. 1. Reduced pup growth and delays in attainment of post weaning developmental landmarks in F2 (auditory canal opening, eye opening) were reported.

In summary, results from pre-natal developmental toxicity studies of butanol in rats, two via inhalation and two via the oral route (drinking water) indicate developmental toxicity at 5 g/kg/day. When butanol was administered 8 weeks premating and further on GD 0-20 increased incidences of dilation of the subarachnoid space and dilation of the lateral and/or third ventricle of the brain in offspring were reported (Sitarek et al., 1994). A more recent study designed with similar dose range to repeat the findings from the Sitarek study, but without the administration during 8 week of premating (Ema et al., 2005), could not repeat these results.

Methyl acrylate
Groups of 25 pregnant rats were exposed to 0, 25, 50 or 100 ppm methyl acrylate (corresponding to approx. 0.089, 0.179, 0.358 mg/L) for 6 hrs/day from days 6 through 20 of gestation (Saillenfait et al., 1999). Marked maternal toxicity was demonstrated at 50 and 100 ppm as pronounced decreases in maternal body weight gain and food consumption over the entire exposure period. Body weights at sacrifice were 90% at 50 ppm and 87% at 100 ppm, as compared to control. In a preliminary study (no data available) at 200 ppm methyl acrylate maternal mortality and a significant weight loss were evident. There were no treatment related increases in embryo/fetal mortality and no fetal malformations were observed in any of the treatment groups. Fetal toxicity, indicated by significantly reduced fetal body weight (83% as compared to control), was observed after exposure to 100 ppm methyl

[18] ECHA dissemination website

acrylate.

A prenatal developmental toxicity study in rabbits was conducted with methyl acrylate according to OECD 414 ([redacted]). Himalayan rabbits were exposed for 5 days/week over a time period of 23 consecutive days (GD 6–28) to methyl acrylate vapours at target concentrations of 0, 5, 15, and 45 ppm (corresponding to approx. 0.0174, 0.0553, 0.1556 mg/L). There were no test substance-related effects on the does concerning food consumption, gross/net body weight, gestational parameters, uterine, placental and lung weights, as well as necropsy observations up to and including a dose of 45 ppm. The test substance caused a severe degeneration and atrophy of the olfactory epithelium in at least one focal area in the nasal cavity (distal levels III and/or IV) at the high-dose level (45 ppm). Methyl acrylate had no adverse effect on prenatal development of offspring at any of the dose levels tested. The study was assigned reliability 1 by the Registrant(s).

Results from a two-generation reproductive toxicity study of methyl acrylate ([redacted]) was included by the Registrant(s) to contribute to the assessment of adverse effects on fertility and sexual function, however, this study should also be included in the total weight of evidence for developmental toxicity. The results of the study are briefly summarized in the section III. 1. There was no developmental toxicity reported in this study.

In summary, based on results from one prenatal developmental toxicity study in rat and one in rabbit, methyl acrylate does not seem to be a developmental toxicant.

Ethyl acrylate

The developmental toxicity of ethyl acrylate was evaluated in Sprague-Dawley rats after inhalation on GD 6 to 20 (Saillenfait et al., 1999). The exposure concentrations were 0, 25, 50, 100, or 200 ppm (corresponding to 0, 0.10, 0.21, 0.41, or 0.82 mg/L). Significant decreases in maternal body weight throughout exposure to 200 ppm were observed (87% as compared to control). Fetal body weights were significantly reduced at 200 ppm (7-8 % lower than control). The study was assigned reliability 2 by the Registrants of the substance.

Pregnant Sprague-Dawley rats were exposed to 0, 50, or 150 ppm of ethyl acrylate (corresponding to 0, 0.21, 0.62 mg/L) during days 6 through 15 of gestation (Murray et al., 1981; IATG 1982). Slight maternal toxicity as evidenced by decreased body weight gain (90% as compared to control, $p<0.05$), decreased food consumption and increased water consumption was noted among rats exposed to 150 ppm of ethyl acrylate. No maternal toxicity was evident in pregnant females exposed to ethyl acrylate at the 50 ppm level. External, internal, and skeletal examination of the pups revealed 3 fetuses from 3 different litters in the 150 ppm group had major malformations (not statistically significant); hypoplastic tail (3), small anal opening, ectopic (2) ovaries (1). The study was assigned reliability 2 by the Registrant(s) of the substance.

In summary, there are some indications of developmental toxicity of ethyl acrylate in rats at 150 ppm with pups displaying major malformations where only slight maternal toxicity is evident in the study by Murray et al (1981). Similar developmental effects were not reported in a later study at higher doses (200 ppm) by Saillenfait et al (1999).

2-Ethylhexyl acrylate:
Pregnant rats were exposed to 2-ethylhexyl acrylate (99.7 % purity) at 0, 50, 75, and 100 ppm (approximately 0.38, 0.56, and 0.75 mg/L) during day 6 to day 20 of gestation (Saillenfait et al. 1999). Dams from the 100-ppm groups showed an absolute weight gain of 24±16 g through the period of exposure, which was lower and statistically significantly different from that of the concurrent control group (42 ± 11 g). Also food intake through the period of exposure of the 100-ppm group was somewhat lower and statistically significantly different in comparison to that of the concurrent control group. Mean fetal body weights were slightly lower in the treated groups, however not statistically significantly different from that of the concurrent control fetuses.

In summary, no developmental toxicity of 2-ethylhexyl acrylate was revealed in this study for concentrations of up to and including 100 ppm.

Tert-butyl acrylate
A study according to OECD 422 of tert-butyl acrylate in rat via inhalation ([redacted]) was included in the read-across assessment of adverse effects on sexual function and fertility, however, the results from this study should also be considered in the total weight of evidence evaluation of developmental toxicity. The highest dose tested (180 ppm) caused excessive maternal toxicity manifested as 20% mortality on GD 18 and GD 20. Live birth index was statistically significantly decreased and the percentage of dead pups and pups cannibalized were statistically significantly increased at this dose. The study was assigned reliability 1 by the Registrants of the substance, but ECHA considers that the interpretation of the results is limited due to inappropriate dose selection.

Registrant(s)' conclusion about the endpoint developmental toxicity
The provided data has been concluded by the Registrant in the following statement: *"n-Butyl acrylate was tested 3 different teratogenicity studies in rats and mice. There were no indications that n-butyl acrylate caused a developmental toxic effect in concentrations or doses which did not cause overt maternal toxicity (incl. death), and the observed embryo-/fetotoxic effects are convincingly of secondary nature.*

Also most of the acrylate category members were tested concerning developmental toxicity in one or two species each. None of these substances induced teratogenicity, again only in high maternal toxic doses signs of embryo/fetotoxicity were described as secondary effect.

Taken together, all data on n-butyl acrylate, the acrylates category, the metabolites n-butanol and acrylic acid, there is enough weight of evidence that n-butyl acrylate is not a teratogen."

Conclusion about the endpoint developmental toxicity
The Registrants have sought to adapt this information requirement using a read-across and weight of evidence approach, and have provided studies conducted with the registered substance, its metabolites and acrylate category members.

The Registrant(s) claim that due to structural similarity and rapid hydrolysis of the category members, toxicity of butyl acrylate could be derived from testing acrylic acid and alcohols associated with the esters. However, as stated in section III.0. above, the analysis of the structural differences of parent compounds and the corresponding metabolites and their impact on the properties and (eco)toxicological profile of the category members is missing. In addition, sufficient information on the

rate and extent of hydrolysis for the parent compound has not been provided. Therefore, the adaptation of the information requirement suggested by the Registrant(s) cannot be accepted and the possibility to predict from the other category members and from metabolites of butyl acrylate to the target substance as required in Annex XI, 1.5. has not been demonstrated.

In addition, according to Annex XI, 1.2., '*There may be sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion.*' When sufficient weight of evidence is available and adequate and reliable documentation have been provided, further testing may be omitted to meet the information requirements. The Registrant(s) have not provided sufficient evidence to demonstrate that the potential developmental toxicity of butyl acrylate can be concluded from the available data.

Therefore, it is considered that the available information does not provide the information required by Annex X, Section 8.7.2. The conditions set out in Annex XI, Sections 1.2 and 1.5 for adaptions of information requirements are not fulfilled mainly with regards to 'being adequate for the purpose of classification and labelling and/or risk assessment.' Consequently there is an information gap and it is necessary to provide information for this endpoint.

Specification of the test
According to the test method EU B.31./OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species. As testing already has been performed in rat with equivocal results, testing in a second species should be performed in accordance with REACH Annex X standard information requirements.

Deviation from the default oral exposure route as recommended in the test guideline requires justification. In the case of butyl acrylate, inhalation is the relevant human exposure (and dermal exposure to some extent) according to the Registrant(s). Repeated inhalation of butyl acrylate has been shown to cause irritating effects to nasal and respiratory mucosa and the eyes at dose levels above 100 ppm in rats. Local irritation may cause stress and reduced body weight in the pregnant animals and may not allow proper evaluation of the relationship between real systemic effects in does with developmental toxicity. In case a substance causes respiratory irritation, an oral route would be more appropriate. This may allow higher systemic dose levels relevant for classification and labelling purposes to be used. The Registrant(s) are therefore requested to perform a prenatal developmental toxicity test via the oral route.
The study should include at least three treatment dose levels and a concurrent control. The highest dose should be selected with the aim to induce some toxicity.

The Registrant(s) in their comments stated that they did not agree with the eMSCA's proposal to perform an additional teratogenicity study in rabbit. The Registrant(s) consider the data on n-butyl acrylate, the acrylates category, the metabolites n-butanol and acrylic acid, as enough weight of evidence that n-butyl acrylate is not a teratogen.

ECHA considers the request of a prenatal developmental toxicity study in rabbit justified based on the following arguments:
1. The available database on n-butyl acrylate indicates a concern for developmental toxicity in form of increased amount of resorptions in one rat study (Merkle and Klimisch, 1983). This concern was not solved in another rat study (Saillenfait et al., 1999).
2. The available study in mouse ([redacted]) is not considered sufficient as

the second species study. The reliability of the study is not assignable since the full study report is not available to the Registrants or the eMSCA. Therefore the validity of the results is questionable. Moreover, mortality was reported at each dose level at or above the limit dose (1000 mg/kg bw/day) which is considered as excessive toxicity. The lowest dose level in the study (100 mg/kg bw/day) was the only dose level without mortality. Therefore, the selected dose spacing is considered to be inappropriate. It is thus concluded that the study is not acceptable.
3. At Annex X level, a prenatal developmental toxicity study (EU B.31., OECD 414), conducted on a second species is a standard information requirement. Omitting a prenatal developmental toxicity study in a second species by adaptation pursuant to Annex X, Section 8.7., Column 2 or pursuant to Annex XI, cannot be justified for butyl acrylate, when taking into account the outcome of the available prenatal developmental toxicity tests and all other relevant available data.. The justification of the read-across is rejected based on the argumentation provided in section III.0.

Conclusion
A prenatal developmental toxicity study of butyl acrylate in rabbit at relevant dose levels is foreseen to generate data that will aid in the evaluation of developmental toxicity and potentially arriving at a conclusion regarding classification according to criteria in Annex I to CLP (Regulation (EC) No 1272/2008). New data may also allow the Registrant(s) to establish an endpoint-specific NOAEL that were not available in the current registration.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision: Pre-natal developmental toxicity study in rabbits, by oral route (test method: EU B.31./OECD 414).

3. Tiered approach strategy for mutagenicity potential assessment (Tiers 1 to 3)

Concern
The available information does not enable the evaluating MSCA to convincingly dismiss the concern for the mutagenic potential of the substance. The available studies have insufficient test design and consequently the readout of the studies is unreliable. Moreover, it is considered that the available information in the current registration does not give adequate basis to read-across to data on mutagenicity of the structural analogues methyl acrylate and ethyl acrylate.

Summary of justification
It is considered that a tiered approach strategy for mutagenicity testing will address the concerns expressed; potential genotoxic effects for workers. Furthermore, the tiered testing strategy will also provide information on CMR properties of the substance. Based on the available data, investigation of chromosome damaging potential is considered a critical first step. Performing of test investigating chromosomal damaging potential *in vitro* using the OECD 487 *In vitro* mammalian cell micronucleus test is therefore requested in Tier 1. Depending on the outcome, further studies may be needed to clarify the concern as required by standard information requirements according to REACH Annex VIII, IX and X to enable a conclusion on the potential mutagenicity of butyl acrylate. *In vivo* testing in somatic cells is triggered by positive in vitro tests and if in vivo test results in somatic cells are positive, the potential for germ cell mutagenicity of the substances should be considered according to Annex IX column 2. Information on mutagenicity is needed for DNEL/DMEL derivation for the critical effects and endpoint specific DNEL/DMEL.

Relevant available data
In vitro gene mutation study in bacteria:

Two bacterial gene mutation tests of butyl acrylate are included in the registration. Both studies were negative but in one of the studies ([redacted]) the test substance was not tested up to cytotoxic concentration (max dose 900 ug per plate or 1000 nL per plate). Test guideline (OECD 417) recommends that test substances that are cytotoxic already below 5 mg/plate or 5 ml/plate should be tested up to a cytotoxic concentration. The study by Zeiger E et al. (1987) is in contrast reliable and thus it is possible to conclude that butyl acrylate is negative for mutagenicity in bacterial gene tests.

In vitro cytogenicity study in mammalian cells:
An in vitro mammalian cell chromosome aberration test of butyl acrylate was performed in CHO cells (NTP 1991a). In presence of metabolic activation no aberrant cells were detected in either of two trials. No cytotoxicity that influenced the read-out was reported (concentrations tested approx. 150-400 ug/ml, cytotoxicity from 298.6 ug/ml, however no data presented). In contrast, the results in one out of two trials in absence of metabolic activation and in presence of some degree of cytotoxicity (unclear to what extent) indicated a weakly positive mutagenic property of butyl acrylate. However, in these two trials there was not an adequate number of cells and concentrations analyzable, according to the Registrant(s)'s report. The study was assigned reliability 2 by the Registrant(s) which interpreted the results to be negative '*since no increase in aberrant cells in concentrations which did not cause strong cytotoxicity were reported and the tested concentrations exceeded the limit concentration of TG 473 of 10 mM /12.8 ug/ml.*' ECHA concludes that the results from this study are not reliable based on the lack of an adequate number of cells and concentrations to be analysed. According to OECD 473, at least three test concentrations (not including the solvent and positive controls) that meet the acceptability criteria (appropriate cytotoxicity, number of cells, etc.) should be evaluated.

Results from an Unscheduled DNA synthesis assay in SHE cells without metabolic activation (Wiegand HJ et al., 1983, 1989) was reported to be negative for butyl acrylate. An identified deficiency of this study was the lack of cytotoxic effects at the highest concentration (or any of doses tested).

A sister chromatid exchange assay in CHO cells (NTP 1991b) was positive (both with and without metabolic activation) for genotoxicity of butyl acrylate at non-cytotoxic concentrations.

In vitro micronucleus study in mammalian cells:
An in vitro micronucleus study in SHE cells (Wiegand HJ et al. 1983, 1989) was negative tested up to 8 mM butyl acrylate. Cytotoxicity was claimed by the Registrant(s) to have been confirmed in pre-tests. However, ECHA notes that the highest concentration used in the in vitro mammalian cell micronucleus test should elicit some cytotoxic effects; and cytotoxicity should be present in main experiment according to the test guideline. The available data does not show any sign of cytotoxicity in the main study up to the top concentration of 8 mM. Moreover, according to the test guideline (OECD 487, 2014), if no precipitate or limiting cytotoxicity is observed, the highest test concentration should correspond to 10 mM, 2 mg/ml or 2 µl/ml, whichever is the lowest. The study is therefore considered to be less reliable and potentially false negative since the selected doses are not demonstrated to be high enough, according to the OECD 487. The study was assigned reliability 2 by the Registrant(s).

In vivo somatic cell genotoxicity study:
In vivo chromosome aberration tests in rat exposed to butyl acrylate via inhalation for 6 h/day for 4 days at 820 ppm (4.3 mg/ml; 1/3 of LC50) was reported to be negative (Engelhardt and Klimisch 1983). There was a slight decrease in mitotic index (24% in males and 14% in female) in bone marrow demonstrating cytotoxicity in target organ. The 4 days

of exposure caused clinical signs of dyspnoea, bloody discharge from eyes and nose and decrease in body weight (6-7 %) indicating that the dose was near the MTD.

ECHA considers that the study suffers from the following limitations in study design:

1) Only one dose was tested which was not limit dose. A minimum of three dose levels generally separated by a factor of 2, but not greater than 4 should be included according to OECD 475.
2) Rats were exposed for 6 hr/day for four days however, there are little data available on the suitability of a repeated-dose protocol for this test according to the test guideline.
3) A positive control is missing and there is no argument for waiving of this.
4) Sampling time was 5 hours post exposure. According to OECD 475, if dose regimens of more than one day are used, one sampling time at up to approximately 1.5 normal cell cycle lengths (normally 12-18 hours) after the final treatment should generally be used.

In conclusion, the deficiencies in study design are considered to severely impact the interpretation of the results and no reliable conclusion can be made from this study.

An in vivo chromosome aberration test was also carried out in hamster exposed to butyl acrylate via inhalation for 6 hr/day for 3 days and 5 h/day for 1 day at 817 ppm (4.28 mg/ml; 65% of LC50) (Engelhardt and Klimisch 1983). There was a slight decrease in mitotic index (31% in males and 7% in female) in bone marrow demonstrating slight cytotoxicity in target organ. The 4 days of exposure caused clinical signs of dyspnoea, disequilibrium, bloody discharge from eyes and nose, mortality of 4/10 animals and decrease in body weight (20 %) (unknown if females or males). MTD was therefore considered to have been reached.

This study has similar limitations in study design as the in vivo rat chromosome aberration study described above (1-4). In addition, at the only dose tested there was mortality and 20% decrease in body weight which is considered as excessive toxicity and the study results are therefore considered to be impacted by systemic toxicity and not reliable.

<u>In vitro gene mammalian cell gene mutation test</u>:
To comply with REACH information requirement, Annex VIII 8.4.3 (2) of in vitro gene mutation study in mammalian cells the Registrant(s) refer to read across to test data from methyl acrylate and ethyl acrylate.

Evaluation of read-across data and weight of evidence for mutagenicity endpoint
In the technical dossier the Registrant(s) provided information with which they sought to fulfil the standard information requirement in Annex IX, 8.7.3.

The Registrant(s) are using grouping and read-across approach and weight of evidence approach for this endpoint and have provided data on n-butyl acrylate ([redacted]; Reininghaus et al., 1991; [redacted], Wiegand HJ 1983, Engelhardt and Klimisch, 1983; Wiegand HJ et al. 1989; NTP 1991; [redacted], Zeiger 1987) and on category members methyl acrylate and ethyl acrylate (Moore et al. 1989, 1991; [redacted]; Moore 1988, 1989; Amtower 1986).

Butyl acrylate
Studies of butyl acrylate for mutagenicity were described in sections above. In addition, the Registrant(s) refer to results from carcinogenicity studies of butyl acrylate to be included in the weight of evidence assessment for mutagenicity.

In a 2-year inhalation study, Sprague-Dawley rats were exposed by whole body exposure 6 hours per day, 5 days a week to 0, 15, 45 or 135 ppm (corresponding to approx. 0, 0.086, 0.258, 0.773 mg/L/day) butyl acrylate. During the first 13 weeks of the study, the concentrations were lower: 0, 5, 15 or 45 ppm. The post observation period was 6 months. Butyl acrylate was shown to have an irritating effect in the area of transition between respiratory and olfactory epithelium in the nasal cavity and in the cornea. A dose-related reserve cell hyperplasia in the transitional region between the respiratory and olfactory nasal epithelium, in part with loss of the functional epithelial component, occurred in all dose groups. Corneal opacification and vascularization occurred at the highest dosage. The changes in the nasal mucosa and cornea proved to be reversible up to a point in the follow-up period. Based upon an examination of the histopathological findings, it was concluded that n-Butyl Acrylate was not carcinogenic in this study.

The dermal carcinogenic potential of butyl acrylate was assessed by applying 25 µL of a 1% (v/v) dilution in acetone (corresponding to approx. 8 mg/kg bw) to the backs of 40 male C3H/HeJ mice three times a week throughout the lifetime of the animals ([redacted]). No biologically significant skin tumors were observed in the group tested with acetone or in the butyl acrylate group. No signs of skin irritation were observed in this study.

Methyl acrylate

Methyl acrylate was tested in an in vitro mammalian cell gene mutation HPRT assay in CHO cells without metabolic activation (Moore et al., 1989b/1991a/1991b). The results were concluded to be negative. The study was assigned reliability 2 by the Registrant(s).

An in vitro Mammalian cell gene mutation TK assay in mouse lymphoma L5178Y cells (according to Clive et al 1979) without metabolic activation of methyl acrylate was concluded to be positive (Moore 1988 /1989b). The study was assigned reliability 2 by the Registrant(s). ECHA notes that the cell survival was 34% at the lowest dose and 16% at the highest dose, moreover, only three concentrations were tested. At least four test concentrations (not including the solvent control and positive control) that meet the acceptability criteria (appropriate cytotoxicity, number of cells) should be evaluated according to the OECD TG for In vitro mammalian cell gene mutation test using the thymidine kinase gene (either OECD 476 or new test guideline OECD 492[19]). The test was not performed according to the test guideline and can thus be considered less reliable.

In a second in vitro Mammalian cell gene mutation TK assay in mouse lymphoma L5178Y cells without metabolic activation methyl acrylate was indicated to have mutagenic properties (Amtower, 1986). However, the Registrant(s) assigned reliability 4 to this study.

Ethyl acrylate

Ethyl acrylate was tested In vitro mammalian cell gene mutation HPRT assay in CHO cells (according to Oberly et al 1987) without metabolic activation (Moore et al 1989a). The results were concluded to be negative for gene mutations and the study was assigned reliability 2 by the Registrant(s). ECHA notes that the lowest dose tested of ethyl acrylate induced high cytotoxicity with only 25%cell survival and therefore considers the dose selection is not appropriate according to the test guideline (OECD 476).

An in vitro Mammalian cell gene mutation HPRT assay in CHO cells (according to Oberly et al., 1987) without metabolic activation (Moore et al., 1989b/1991a/1991b) was concluded to be negative by the Registrant(s) and reliability 2 was assigned.

[19] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

In vitro Mammalian cell gene mutation Mouse lymphoma L5178Y cells (according to Turner et al 1984) without metabolic activation (Moore et al., 1989a) was concluded to be positive and reliability was assigned to 2 by the Registrant(s). The study by Moore et al., 1989 demonstrated that ethyl acrylate has the potential to induce gene mutation at a concentration where survival was 60%. Mainly small colonies were formed (148/37 small/large colonies), which is indicative of a clastogenic mechanism.

A second in vitro Mammalian cell gene mutation TK assay in mouse lymphoma L5178Y cells (according to Clive and Spector 1975) with and without metabolic activation of ethyl acrylate was also concluded to be positive ([redacted]). Reliability 2 was assigned by the Registrant(s). The increase in mutants was reported to be only observed at cytotoxic doses; however, mutation frequency and survival were not reported for each dose tested. Colony size was not evaluated in this study.

A third in vitro Mammalian cell gene mutation TK assay in mouse lymphoma L5178Y cells without metabolic activation (Amtower et al., 1986) with reliability 4 (as assigned by the Registrants) was also reported to be positive for mutagenicity of ethyl acrylate.

Registrant(s)' conclusion about the endpoint mutagenicity
The provided data has been concluded by the Registrant(s) in the following statement:
'We don't agree with the eMSCA that any further testing is needed to evaluate a possible genotoxicity potential of n-butyl acrylate.

- *In vitro tests did not show genotoxic effects in non-cytotoxic concentrations.*
- *Two reliable in vivo micronucleus tests, using the most appropriate route of exposure, tested up to severe toxic (lethal) concentrations and clear indications of systemic availability of the product / metabolites did not show any cytogenetic effect.*
- *The chemical class and the metabolic degradation products give no suspicion that n-butyl acrylate has a genotoxic potential in vivo.*
- *Two reliable long-term carcinogenicity tests did not show any indication of a possible carcinogenic effect.*

Overall n-butyl acrylate gives no indication to have a genotoxic potential.'

Conclusion about the endpoint mutagenicity
The Registrant(s) have sought to adapt this information requirement using a read-across and weight of evidence approach, and have provided studies conducted with the registered substance, its metabolites and category members.

The Registrant(s) claim that due to structural similarity and rapid hydrolysis of the category members, toxicity of butyl acrylate could be derived from testing acrylic acid and alcohols associated with the esters. However, as stated in section III.0. above, the analysis of the structural differences of parent compounds and the corresponding metabolites and their impact on the properties and (eco)toxicological profile of the category members is missing. In addition, sufficient information on the rate and extent of hydrolysis parent compound has not been provided. Therefore, the adaptation of the information requirement suggested by the Registrants cannot be accepted and the possibility to predict from the other category members and from metabolites of butyl acrylate to the target substance as required in Annex XI, 1.5. has not been demonstrated.

In addition, according to Annex XI, 1.2., *'There may be sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion.'* When sufficient weight of

evidence is available and adequate and reliable documentation have been provided, further testing may be omitted to meet the information requirements. The Registrant(s) have not provided sufficient evidence to demonstrate that the potential mutagenic property of butyl acrylate can be concluded from the available data.

Two in vitro gene mutation studies in bacteria indicated negative results for mutagenicity of butyl acrylate ([redacted]; Zeiger et al., 1987). Additionally, two in vitro studies of butyl acrylate in Syrian hamster embryo fibroblasts (SHE-cells) demonstrated negative results for genotoxicity: a micronucleus test (Wiegand HJ 1983) and a DNA damage and repair assay, unscheduled DNA synthesis (Wiegand HJ et al., 1989). In contrast, one in vitro chromosome aberration assay indicating weakly positive results in Chinese hamster ovary (CHO) cells without metabolic activation, and one in vitro sister chromatid exchange assay demonstrating positive results for genotoxicity in CHO cells both with and without metabolic activation (NTP 1991) are available. Chromosome damaging properties of butyl acrylate was also investigated in vivo in rat and hamster (in vivo mammalian bone marrow chromosome aberration test) after inhalation suggesting no mutagenicity in somatic cells (Engelhardt and Klimisch, 1983). However, in this case as stated above, also the data from the in vivo test are considered not reliable.

In the technical dossier of the registration, neither *in vitro* (mammalian cells) nor *in vivo* gene mutation studies of butyl acrylate are available. Instead, read across to methyl acrylate and ethyl acrylate data was done for *in vitro* gene mutation studies in mammalian cells. The read-across data showed that at concentrations causing a positive response in mutant frequency, relatively more small than large colonies were formed. This is indicative of a clastogenic potential of the test substances methyl and ethyl acrylate. However, the gene mutation read-across data are considered as equivocal and uncertainties were identified regarding the studies. The read-across was not justified by the Registrant(s) and no assessment of the appropriateness to use read-across for this endpoint was provided in the technical dossier. However, the Registrant(s) have provided further information to justify the acrylate category and the read-across approach to fill existing data gaps. The Registrant(s) also refer to existing data on the products of hydrolysis of butyl acrylate: acrylic acid and butanol to be included in the overall weight of evidence, however no data have been included to support this. The data base on the source chemicals is not conclusive to support a read-across within a chemical category of acrylates for the end point mutagenicity. Although the data are equivocal, the read-across was not accepted as stated above and explained in section III.0. These data are therefore not taken into account in the assessment of gene mutation for the registered substance.

Finally, regarding the impact of two negative carcinogenicity studies in the total weight of evidence for gene toxicity ECHA considers that mutagenicity data cannot be replaced with data from carcinogenicity studies and further considers carcinogenicity studies not sensitive enough to detect mutagenic potential.

Therefore, ECHA considers that the available information does not provide the information required by Annex VIII, 8.4. The conditions set out in Annex XI, Sections 1.2 and 1.5 for adaptions of information requirements are not fulfilled mainly with regards to 'being adequate for the purpose of classification and labelling and/or risk assessment.' Consequently there is an information gap and it is necessary to provide information for this endpoint.

Tiered approach strategy for mutagenicity potential assessment
ECHA requests to evaluate the genotoxicity potential of butyl acrylate following a tiered approach.

Tier 1:

An *in vitro* mammalian cell micronucleus test(test method: OECD 487) with the addition of chromosome centromere labelling method (e.g. FISH, CREST) is required in order to cover the concern for mutagenicity due to the inconclusive results observed in available studies of butyl acrylate. The addition of the centromere labelling method may also enable the distinction between a suspected clastogenic or aneugenic mechanism of butyl acrylate. This information will have importance when considering *in vivo* testing, which is triggered according to REACH information requirements in Annex IX and X if the *in vitro* test is positive, and for risk assessment purposes.

Tier 2:

a) In case of a negative result in In vitro mammalian cell micronucleus test: an In vitro mammalian cell gene mutation test using the thymidine kinase gene (new test guideline OECD 492)[20] is requested to enable a conclusion on the in vitro mutagenicity of butyl acrylate; this test is triggered according to REACH Annex VIII 8.4.3. The information derived from this test is considered necessary by ECHA to clarify the potential of butyl acrylate to induce in vitro gene mutations.
b) In case of positive result in the *In vitro* mammalian cell micronucleus test mainly demonstrating aneuploidy, the Mammalian erythrocyte micronucleus test (B.12./OECD 474) in rats via the oral route with appropriate dose levels considering cytotoxicity and target organ availability of the substance.
c) In case of positive result in the In vitro mammalian cell micronucleus test and if the chromosome centromere staining demonstrates both structural and numerical chromosomal changes, the Registrant(s) shall perform a combined in vivo Mammalian erythrocyte micronucleus test (EU B.12./OECD 474) and an In vivo mammalian alkaline comet assay (OECD 489) in rat via oral gavage. The combined test has to be performed in accordance with OECD TG 489 and EU B.12/OECD TG 474 with an adequate treatment schedule for the combined assay (i.e. including a third dose administered on the 3rd day) as described e.g. by Bowen et al., 2011.
d) In case of clastogenic effect in the In vitro mammalian cell micronucleus test an In vivo mammalian alkaline comet assay (OECD 489) in rats via oral gavage. DNA damage shall be assessed in forestomach, glandular stomach, and liver.

At Tier 1, Registrant(s) are requested perform an in vitro mammalian cell micronucleus test (OECD 487) with the addition of a chromosome centromere labelling method (e.g. FISH, CREST) as is mentioned in paragraph 4, 5 and 42 of the OECD 487 and paragraph 42 of OECD 474. This is considered justified when there is an increase in micronucleus formation to be able to further determine if the increase was the result of clastogenic and/or aneugenic events and will allow the Registrant(s) to decide on the most appropriate follow-up test at Tier 2. The standard OECD 487 test, i.e. without centromere labelling, does not allow the distinction between clastogenic and aneugenic events.

At Tier 2, in case of negative results from the in vitro mammalian cell micronucleus test (OECD 487), the Registrant(s) are requested to perform the In vitro mammalian cell gene mutation test using the thymidine kinase gene (test method:new test guideline OECD 492)[21]. This follows the testing strategy in ECHA guidance on

[20] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm
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Information Requirements and Chemical Safety Assessment, chapter R.7a (Version 2.4, 2014) and is considered justified to further investigate mechanism on mutagenicity and may give an indication if the substance has the potential to induce point mutations or structural chromosomal aberrations.

At Tier 2, in case of positive result in the *In vitro* mammalian cell micronucleus test, the Registrant(s) are requested to perform either a Mammalian erythrocyte micronucleus test, a combined in vivo Mammalian erythrocyte micronucleus test (EU B.12./OECD 474) and an In Vivo mammalian alkaline comet assay (OECD 489), or an In Vivo mammalian alkaline comet assay as specified in Section II, subject to the conditions presented in the Section III.

The Mammalian erythrocyte micronucleus test shall be performed in rats via the oral route. This will allow higher doses to be administered and systemically available. The animals should be given 2 or more treatments (single treatments can be administered, if scientifically justified according to the updated OECD 474 guideline default schedule), and samples of bone marrow should be taken at least twice, starting not earlier than 24 hours after treatment, but not extending beyond 48 hours after treatment. The highest dose should produce some indication of toxicity in the bone marrow (e.g. a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow). It is noted that evidence has to be provided for the substance or its metabolites reaching the bone marrow to be able to make a robust conclusion on the lack of in vivo clastogenicity based on the result of the test. ECHA notes that the relevant human exposure is inhalation whereby testing by the inhalation route could be considered appropriate. However, considering the irritating properties of the substance as well as its reactivity, the upper respiratory tract (i.e. the first site of contact) will most likely be the main target of the substance and thus the tissue to be analysed in a comet assay as site of contact. However, there is currently a technical difficulty associated with the study of this tissue (i.e. nose epithelium) in the comet assay which might prevent the validation of the data generated by such a study. An alternative tissue exposed via inhalation could be considered, i.e. the lung. However, it is expected that this second site of contact tissue will be exposed to a lower dose considering that a fraction of the substance will have reacted at the first site of contact (i.e. upper respiratory tract) given the solubility and high reactivity of the registered substance. Therefore, ECHA considers that testing by inhalation route will not provide relevant data to address the concern identified *in vitro*. The alternative route of administration is the oral route. Based on the above, ECHA considers that performing the comet assay by the oral route is more appropriate for this substance.

TheIin vivo mammalian alkaline comet assay in accordance with OECD 489 shall be performed in rats via oral gavage. DNA damage shall be assessed in forestomach, glandular stomach (initial site of contact) and liver (metabolic active tissue and slowly dividing tissue). Sampling time 2-6 hours after the last treatment.

The combined in vivo Mammalian erythrocyte micronucleus test (EU B.12./OECD 474) and an In vivo mammalian alkaline comet assay (OECD 489) shall be performed in rats via oral gavage. The test has to be performed in accordance with OECD 489 and EU B.12./OECD 474 with an adequate treatment schedule for the combined assay (i.e. including a third dose administered on the 3rd day) as described e.g. by Bowen et al., 2011. DNA damage shall be assessed in forestomach, glandular stomach (initial site of contact) and liver (metabolic active tissue and slowly dividing tissue).

The test shall be performed by using the following tissues: liver as primary site of xenobiotic metabolism, and both forestomach and glandular stomach as sites of direct contact. The request of testing in both forestomach and glandular stomach is justified by the need to address the uncertainty, associated with the administration by oral gavage, on the actual first site of contact. It is particularly important to address the concern on potential genotoxic effects at the first site of contact for this highly reactive substance.

Tier 3:

In case of a positive result in the In vitro mammalian cell gene mutation test using the thymidine kinase gene (new test guideline OECD 492)[22], the TGR (OECD 488) in mice or rats by inhalation or via oral gavage, or In vivo mammalian alkaline comet assay (OECD 489) in rats via oral gavage shall be conducted. In accordance with Annex X, column 2, point 8.4 of the REACH Regulation and the ECHA guidance on Information Requirements and Chemical Safety Assessment, chapter R.7a (Version 2.4, 2014), an *in vivo* test for gene mutations in mammalian cells is the appropriate method to follow up the *in vitro* gene mutation positive results. In line with the current state of science, the TGR test is the method of choice for this endpoint and can detect mutagenic effects in many target tissues. Additionally, the *in vivo* Comet Assay is considered a useful genotoxicity test in terms of its sensitivity both to substances which cause gene mutations and structural chromosomal aberrations and it can detect effects in many target tissues (EFSA 2011, ECHA guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7a (Version 2.4, 2014)). ECHA considers that TGR and Comet Assay are both suitable methods to follow up the concern for gene mutations in somatic cells. Furthermore, Comet Assay is suitable to follow up mutagenic potential via chromosomal aberrations in somatic cells. Therefore, ECHA gives the option to the Registrant(s) to perform either of the two methods at Tier 3. According to the strategy reflected in the legal text, *in vivo* testing for somatic cells is triggered by positive *in vitro* tests. If results of testing in somatic cells are positive, germ cell testing would have to be considered. With regard to TGR, ECHA noted that if results of testing in somatic cells were positive, germ cell mutagenicity would have to be considered. Thus, with a view to avoid unnecessary animal testing, the need to include the sampling of germ cells for conditional analysis in case of positive test results in somatic cells is emphasised. Currently, the *in vivo* Comet Assay is not officially validated for the assessment of DNA damage in germ cells but only for the use in somatic cells (cf. OECD 489). As a consequence, if the Comet Assay is chosen by the Registrant(s) they need to be aware that a positive result in somatic cells in this test would probably indicate the need for further animal testing either in toxicokinetic studies or more likely in a TGR assay employing germ cells, which would be considered in the follow-up pursuant to Article 46(3) of the REACH Regulation. If the Registrant(s), after review of the available database, conclude that there is some likelihood that the substance or its metabolites may reach germ cells, they should consider the TGR test as a first study of choice, as this test (if performed following the test specifications below) allows to assess the potential mutagenic effects both in somatic and germ cells, the latter within the same study conditionally of positive results in somatic cells.

At Tier 3, Registrant(s) are requested to perform either a Transgenic rodent somatic and germ cell gene mutation assay (TGR) or a Comet Assay as specified in Section II, subject to the conditions presented in the Section III. In choosing the appropriate

[22] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

testing strategy the Registrant(s) shall place due weight on animal welfare considerations, i.e. they shall choose the strategy which, based on a thorough review of the data available, is expected to use the least number of experimental animals.

The TGR test shall be conducted in mice or rats treated for 28 days via inhalation or via oral gavage. The choice of the tissues is justified for investigating a tissue at the site of contact (nasal tissue for inhalation route, forestomach and glandular stomach for oral route), a tissue characterised by slow proliferation and strong metabolic activity (liver) and a tissue with rapid proliferation (bone marrow). Tissues shall be harvested three days after the cessation of the treatment.
Mutation frequency shall be assessed in nasal tissue or forestomach and glandular stomach depending on the route of administration, liver and bone marrow. Germ cells from testes shall be sampled and stored. Cells shall be sampled from seminiferous tubules in addition to spermatozoa from the vas deferens/cauda epididymis. The germ cells shall be analysed for mutation frequency only in the case where positive test results are obtained for any of the somatic tissues.

Regarding the route of administration, inhalation is the most relevant human exposure and will allow investigation of effects in the relevant site of contact tissues (nasal tissue). The Registrant(s) are also given the option to perform the study via oral gavage. Due to the irritative properties of the substance, the oral route may allow higher doses to be administered and systemically available. In addition, this administration route is considered as being more technically feasible compared to inhalation.

As for the relevant site of contact tissues, nasal tissue for inhalation route and forestomach and glandular stomach for oral route are considered to be the most appropriate. An alternative site of contact tissue exposed via inhalation could be the lung. However, it is expected that this second site of contact tissue will be exposed to a lower dose considering that a fraction of the substance will have reacted at the first site of contact (i.e. upper respiratory tract) given the solubility and high reactivity of the registered substance.

The Comet Assay shall be conducted as described above in Tier 2.

Conclusion
Generated data from *in vivo* studies may allow a DMEL to be determined that should be used to better target risk management measures. Moreover, information derived from the requested tests may also allow to determine whether the substance acts through an aneugenic mechanism. Aneugens are generally considered to have a threshold effect, whereas clastogens are considered in most cases not to have a threshold effect. For non-threshold mutagens (without information on *in vivo* carcinogenicity) the Registrant(s) is required to carry out a qualitative assessment of the likelihood that effects are avoided, when implementing the exposure scenarios to ensure that appropriate risk management measures (RMMs) and operational conditions (OCs) are in place. In addition, an evaluation of the new data and comparison with CLP criteria in Annex I to CLP (Regulation (EC) No 1272/2008) for hazard assessment may lead to the initiation of a harmonized classification of mutagenicity as a risk management option.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following studies using the registered substance subject to this decision:

Tier 1:

In vitro mammalian cell micronucleus test (test method: B.49./OECD 487) with the addition of a chromosome centromere labelling method (e.g. FISH, CREST)

Tier 2:

a) In case of a negative result in In vitro mammalian cell micronucleus test: the In vitro mammalian cell gene mutation test using the thymidine kinase gene (test method: new guidelineOECD 492)[23];
b) In case of positive result in the In vitro mammalian cell micronucleus test mainly demonstrating aneuploidy: the Mammalian erythrocyte micronucleus test (test method: B.12./OECD 474) in rats via the oral route;
c) In case of positive result in the In vitro mammalian cell micronucleus test and if the chromosome centromere staining demonstrates both structural and numerical chromosomal changes, the Registrant(s) shall perform a combined in vivo Mammalian erythrocyte micronucleus test (EU B.12./OECD 474) and an In vivo mammalian alkaline comet assay (OECD 489) in rat via oral gavage. The combined test has to be performed in accordance with OECD 489 and EU B.12./OECD 474 with an adequate treatment schedule for the combined assay (i.e. including a third dose administered on the 3rd day) as described e.g. in Bowen et al., 2011;
d) In case of clastogenic effects in the In vitro mammalian cell micronucleus test, In vivo mammalian alkaline comet assay (OECD 489) in rats via oral gavage. DNA damage shall be assessed in forestomach, glandular stomach and liver;

Tier 3:

In case of a positive result in the In vitro mammalian cell gene mutation test using the thymidine kinase gene (new test guideline OECD 492)[24]: the Transgenic rodent somatic and germ cell gene mutation assay (TGR, OECD 488) in mice or rats via inhalation or via oral route. The test shall be conducted in mice or rats treated for 28 days via inhalation or via oral gavage. Tissues shall be harvested three days after the cessation of the treatment. Mutation frequency shall be assessed in nasal tissue, liver and bone marrow if inhalation is chosen or in forestomach, glandular stomach, liver and bone marrow if oral route is chosen. Germ cells shall be sampled and stored. Cells shall be sampled from seminiferous tubules in addition to spermatozoa from the vas deferens/cauda epididymis. The germ cells shall be analysed for mutation frequency only in the case where positive test results are obtained for any of the somatic tissues;
OR

[23] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm
[24] The guideline is currently a draft. Final adoption of the guideline is expected by September 2015. The adopted guideline will be published on OECD website: http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm

In vivo mammalian alkaline comet assay (OECD 489) in rat via oral gavage. DNA damage shall be assessed in forestomach, glandular stomach and liver. Points to be considered by the Registrant(s) in choosing which of the two above tests they should perform are given in section III above.

It is noted that the deficiencies in study design of the in vivo chromosomal aberration test (Engelhardt and Klimisch, 1983) is considered to severely impact the interpretation of the results and no reliable conclusion can be made from this study based on the reported information. Specifically, the positive controls data is considered necessary to evaluate reliability of the study as performed with several deviations from the guideline. This information is not reported in IUCLID and CSR. If available, the Registrant(s) may use this information to re-evaluate the reliability of the study and may consider using the data in a weight of evidence approach before performing the in vitro micronucleus test and the following in vivo tests as requested in mutagenicity testing strategy.

Notes for consideration by the Registrant(s):
Once the information requested by this decision is available in the registration dossiers, the evaluating MSCA will be in a position to reassess the situation and on the basis of that assessment they will decide on the need to request further information in order to examine any (remaining) concern pursuant to Article 46(3) of the REACH Regulation. In case of a positive result of butyl acrylate in somatic cells *in vivo*, potential of germ cells mutagenicity should be considered.

It is pointed out that in case of a positive *in vivo* test result in Tier 2 or 3, the Registrant(s) would need to determine whether C&L of butyl acrylate as a mutagen is justified in accordance with Regulation (EC) 1272/2008 (CLP).

4. Further information regarding derivation of dermal DNEL

In the derivation of dermal DNELs for sensitisation the Registrant(s) have disregarded the aspect of intraspecies variation by omitting the application of an AF correcting for this uncertainty. Butyl acrylate is a weak sensitizer (EC = 11.2% w/v) and therefore RMMs and OCs for moderate hazard (table E.3-1, ECHA Guidance on information requirements and chemical safety assessment, Part E: Risk Characterisation, 2012) should be implemented. Moreover, the Registrant(s) have determined the DNEL to be 0.28 mg/cm^2 based on the EC3-value ([redacted]) and applying an AF of 10 for intraspecies differences and no AF for interspecies differences. According to ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8 (Version 2.1, 2012) EC3 value can be considered as the LOAEL for induction, or used as a surrogate for the NOAEL. Moreover, EC3 data generally correlate well with human skin sensitisation thresholds derived from historical predictive testing; however there are cases where this correlation is poor and the two values may differ by 10-fold or more. In view of this variation, the default AF of 10 for interspecies variation should be used, unless there is evidence (e.g. from a close analogue of the substance in question) of good correlation between the EC3 and human NOAEL/LOAEL. Therefore, on a case by case basis the interspecies AF could be lowered. In this regard, the Registrant(s) have failed to motivate the reason not to use an AF of 10 for interspecies variation for deriving dermal DNELs for sensitisation of butyl acrylate. Using an AF of 10 for interspecies variation will lower the current dermal DNEL(s) 10-fold to 0.028 mg/cm^2.

As an alternative to adjusting of dermal DNEL taking into account interspecies variation, the Registrant(s) have in their comments proposed to perform a qualitative assessment for dermal effects instead of a quantitative assessment. ECHA agree that setting a revised DNEL for sensitisation and comparing it with exposure is difficult, since no models exist to

do that. Dermal exposure is a stochastic process, the challenge being highly variable and not equally dispersed over the skin surface. A metric based on average exposure over a default skin area is therefore not applicable unless, for instance the substance is applied within a cream and purposely evenly spread over the skin - which in this case it is not. Long term dermal exposure is event driven and will be a very wide distribution and unpredictable. For these reasons, ECHA accepts the Registrants proposal to perform a qualitative assessment for dermal effects instead of a quantitative assessment.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to provide an improved qualitative assessment to demonstrate the likelihood that skin sensitisation effects are avoided when implementing the exposure scenarios.

IV. Registrant(s) comments to deadlines

Following an ECHA PfA the deadlines to provide the requested information under point 2, 3 Tier 3, and 4 were extended. In their comments to the PfAs the Registrant(s) generally stated that the deadlines for providing the requested information are too short. However, as they did not further substantiate these general statement ECHA did not consider further extension of the deadlines as necessary.

V. Adequate identification of the composition of the tested material

In relation to the required experimental stud(y/ies), the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the test(s) must be shared by the Registrant(s).

VI. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at http://echa.europa.eu/datasharing_en.asp.

If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrant(s) to perform the stud(y/ies) on behalf of all of them.

http://www.echa.europa.eu/regulations/appeals

VII. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal

procedure can be found on the ECHA's internet page at http://echa.europa.eu/appeals/app_procedure_en.asp. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

[redacted]
Leena Ylä-Mononen, Director of Evaluation

Annex 1: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

Annex 2

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