ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 11 December 2018

Addressee: [redacted]

Decision number: CCH-D-2114453510-59-01/F
Substance name: 3-methoxybutyl acetate
EC number: 224-644-9
CAS number: 4435-53-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 22/05/2017
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

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

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

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

3. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

4. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance, provided that both studies requested under 2. and 3. have negative results;**

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

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

You have to submit the requested information in an updated registration dossier by ***18 December 2020***. You also have to update the chemical safety report, where relevant.

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

This decision does not address the information requirement of the Extended one-generation reproductive toxicity study according to Annex X, Section 8.7.3. of the REACH Regulation.

Appeal

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

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

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

Grouping of substances and read-across approach

You have sought to adapt the information requirements listed above by applying a read-across approach in accordance with Annex XI, Section 1.5. According to Annex XI, Section 1.5., two conditions must be fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances. This hypothesis explains why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis- (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

i. Description of the grouping and read-across approach proposed by you

You consider to achieve compliance with the REACH information requirements for the registered substance methoxybutyl acetate using data of structurally similar substances 3-methoxybutan-1-ol (EC No 219-741-8) (hereafter 'source substance 1') and butane-1,3-diol (EC No 203-529-7) (hereafter 'source substance 2').

You have provided a read-across documentation as a separate attachment in the registration.

You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group: the substances are expected to have similar effects via biotranformation of one substance to another. More specifically, you expect that the registered substance will metabolise (via hydrolysis) to give source substance 1, and you expect that the source substance 1 will be further metabolised (via demethylation) to source substance 2. Furthermore, you consider that the registered substance as well as the two source subtances show similar lack of toxicity in the available studies on these substances.

As an integral part of this prediction, you propose that the source and registered substance(s) have similar properties for the above-mentioned information requirements. ECHA considers that this information is your read-across hypothesis.

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

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

Toxicokinetics

One important aspect in establishing that substances have similar effects or follow a regular pattern is the comparison of absorption, distribution, metabolism and elimination of source and target substances. This allows assessing the qualitative and quantitative internal systemic exposure of the test organism when exposed to source and target.

ECHA notes the following observations:

a. You have not provided any studies showing that the registered substance undergoes hydrolysis to source substance 1 and you have not provided information on the rate at which such hydrolysis occurs. In addition, you have not provided any studies showing that source substance 1 undergoes demethylation to source substance 2 and the rate at which such demethylation occurs. Therefore, your hypothesis is not adequately substantiated.

b. Instead of studies on the metabolism source or target substances, your justification document refers to toxicokinetic information from other substances, specifically methoxyethanol, and n-butyl acetate. You cite this information to support your hypothesis that source substance 1 undergoes hydrolysis to the registered subtsance. Specifically, you state the following "In support of the above, detailed metabolism studies on a methoxyethanol (a primary alcohol) closely structurally-related to 3-

methoxybutan-1-ol (Source Substance, Metabolite 1) have been provided by Sumner et al. (1992, 1995)".

In addition, you state that

"Besides the generally recognized metabolic pathways for linear and branched chain alcohols and their acetyl and ester conjugates discussed above, specific information on the rate of conversion of 3-methoxybutyl acetate is provided by metabolism studies with n-butyl acetate. Specifically, n-Butyl acetate is readily hydrolyzed to acetic acid and n-butyl alcohol in the blood, liver, small intestine and respiratory tract, as shown in a number of in vitro experiments using homogenates (Longland et al., 1977; Dahl et al, 1987). Studies with n-butyl acetate added to blood samples from human male volunteers and female rats indicated hydrolysis half-lives for conversion of the acetate to the alcohol were 4 and 12 minutes, respectively (Essig et al., 1989, WHO). As such, the conversion of 3-methoxybutyl acetate (Target Substance) to 3-methoxy-1-butanol (source substance) is expected to be rapid in both rodents and humans. The rapid transformation of the Target Substance to the source substances supports read-across of toxicological data from each of the substances to the other substances, because humans will be exposed to all three chemicals when exposed to the parent (target) substance and toxicity studies reflect exposure to the dosed chemical as well as all of its metabolites."

Regarding the hypothesised demethylation, the only supporting information provided is the following statement

"In general, open chain aliphatic ethers undergo O-dealkylation to yield the corresponding aldehyde and alcohol, followed by complete oxidation to the fatty acid pathway and tricarboxylic acid/Krebs cycle (Krantz and Carr, 1969)."

However, your dossier does not include any endpoint study records for these particular studies, and you have not provided sufficient details of these studies to allow ECHA to assess the adequacy of these studies. Furthermore, you have not provided an adequate justification explaining why information on the toxicokinetics of such substances would allow the prediction of the metabolism of the source and target substances, or the rate of the metabolism.

c. Finally, you refer to a number of assessments by WHO, RIFM, and EFSA, that discuss the metabolism of branched chain alcohols and esters of such alcohols. You note that

"Although not specifically included in the groupings of primary alcohols (and related esters and acetyls that are rapidly metabolized to alcohols) evaluated by WHO, RIFM, and EFSA for flavourings, fragrances, and feed additives, respectively, 3-methoxybutyl acetate and its proximate metabolites (i.e., 3-methoxy-1-butanol and butane-1,3-diol) clearly belong to these groupings."

ECHA notes that although these assessments address several alcohols, they do not include methoxy substituted alcohols, with the exception of the RIFM assessment, which includes a single methoxy substituted alcohol. The RIFM report states the following regarding the metabolism of this particular substance "One member contains a methoxy group. Metabolism studies are lacking for this compound, however, a methoxy group is

enzymatically not readily cleaved and if it were so, another primary alcohol group would be formed." ECHA considers that you have not taken the structural differences between the registered substance and the substances included in these assessments into account in reaching your conclusion that these substances "clearly belong to these groupings". As noted by you, the registered substance and the source substance are not included in any of these assessments. Therefore, it is not possible to conclude that the results of these assessments provide sufficient information on the metabolism of the source and target substances.

ECHA concludes that you did not adequately address important aspects such as the toxicokinetics of the registered substance and its metabolic fate / (bio)transformation and the resulting possible difference in the metabolite profile, as compared with the source substances. Therefore, it is not possible to verify the quantitative and qualitative basis of conversion between source and target substances, and the relationship to determining the toxicological properties of the registered substance. In the absence of such information there is not an adequate basis for predicting the properties of the registered substance from the data obtained with the source substance.

In addition to your arguments regarding the metabolism of the source and target substance, you consider that the substances display similar toxicological properties. However, toxicological similarity in one or multiple endpoints does not necessarily lead to predictable or similar human health properties in other endpoints. Given the deficiencies highlighted above regarding the toxicokinetics of the source and target substances, ECHA considers that you have not established why a prediction for a human health property is reliable. Thus toxicological similarity on certain endpoints is not sufficient to enable the prediction of other human health properties of a substance.

On that basis, the requirement of Annex XI, Section 1.5., that human health effects may be predicted from data on some endpoints for reference substance(s) within the group, has not been met.

As described above, further elements are needed to establish a reliable prediction for a toxicological or ecotoxicological property, based on recognition of the structural similarities and differences between the source and registered substances. This could be achieved (if it is possible) by a well-founded hypothesis of (bio)transformation to a common compound(s), or that the registered and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health properties.

In your comments on the draft decision you have a provided an improved read-across justification. ECHA notes that you have taken into account a number of the above shortcomings identified by ECHA.

According to the improved read-across justification the hypothesis is for an analogue approach based on two aspects:

- (Bio)transformation of one substance into another (i.e. Scenario 1 of ECHA's RAAF): i.e. target substance transforms by hydrolysis of the acetate group to the source substance 1 (3-methoxybutan-1-ol) which transforms by demethylation of the methoxide group to source substance 2 (butane-1,3-diol).
- Different substances with the same effects (Scenario 2 of the RAAF); i.e. the target substance, source substance 1, and source substance 2 have similar toxicological properties (i.e. 'low toxicity').

ECHA notes that in respect of the claimed (bio)transformation, the basis for read-across between the registered substance and source substance 1 seems plausible (enzymatic deacetylation), but is not supported by data on the registered substance and source substance 1.The possiblity that there is significant exposure to the parent substance has not been excluded. ECHA considers that the second biotransformation of demethylation of source substance 1 to source substance 2 has not been established as plausible. Ethers are chemically stable, and the existence of a demethylating activity, its location and capacity, would have to be empirically demonstrated. It is possible that the results of such a study would show that source substance 2 is not a significant metabolite of the registered substance and source substance 1.

In view of the above, ECHA notes that to support your read-across justification you would need to provide data, such as toxicokinetics data confirming the claimed (bio)transformations, on the registered and source substances. In order to allow you the possibility to generate such data, ECHA has extended the deadline of the decision from 18 to 24 months.

Finally, ECHA notes that the deadline of the separate decision CCH-D-2114453554-47-01/F on a compliance check on the source substance 1 (3-methoxybutan-1-ol (EC No 219-741-8)) has also been extended from 30 to 36 months.

iii. Conclusion on the read-across approach

The adaptation of the standard information requirements sub-chronic toxicity study (90-day), in vitro gene mutation study in bacteria, screening for reproductive/developmental toxicity, and pre-natal developmental toxicity study in the technical dossier is based on the proposed read-across approach examined above. ECHA does not consider the read-across justification to be a reliable basis to predict the properties of the registered substance for the reasons set out above. Thus, the adaptation does not comply with the general rules of adaptation as set out in Annex XI, 1.5. Therefore, ECHA rejects all adaptations in the technical dossier that are based on Annex XI, 1.5.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

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

You have provided a total of seven endpoint study records on repeated dose toxicity in your registration dossier. These are:

1) A short-term repeated dose toxicity study on the registered substance, via the inhalation route. The duration of the study was 4 weeks, and was conducted in rats, guinea pigs, cats and dogs. No particular test guideline was followed, and the study

was not performed according to GLP. You have assigned this study a reliability score of of 2 (reliable with restrictions) ([redacted] 1964).

2) A pre-natal developmental toxicity study on the registered substance, via the oral route. The study was performed according to OECD TG 414. The study was performed with only a limit dose of 1000 mg/kg/day. ([redacted] 1997)
3) A multi-generation study on the analogue substance butane-1,3-diol (source substance 2), via the oral route. The duration of the study is described as "approximately 2 years", and was conducted in rats. The study was conducted with a guideline "equivalent or similar to OECD TG 416" with deviations, and the GLP status of the study is not known. You have assigned this study a reliability score of 2 (reliable with restrictions)([redacted] 1981)
4) A short-term repeated dose toxicity study on the analogue substance 3-methoxybutan-1-ol (source substance 1), via the inhalation route. The duration of the study was 4 weeks, and was conducted in rats, guinea pigs, cats and dogs. No particular test guideline was followed, and the study was not performed according to GLP. You have assigned this study a reliability score of 2 (reliable with restrictions) ([redacted] 1964).
5) A chronic toxicity study on the analogue substance butane-1,3-diol (source substance 2), via the oral route. The duration of the study was 2 years, and was conducted in rats. No particular test guideline was followed, and the study was not performed according to GLP. You have assigned this study a reliability score of 2 (reliable with restrictions)([redacted] 1967)
6) A chronic toxicity study on the analogue substance butane-1,3-diol (source substance 2), via the oral route. The duration of the study was 2 years, and was conducted in beagle dogs. No particular test guideline was followed, and the study was not performed according to GLP. You have assigned this study a reliability score of 2 (reliable with restrictions)([redacted] 1967)
7) A sub-chronic toxicity study on the analogue substance butane-1,2-diol (source substance 2). The duration of the study was 30 weeks, and was conducted in rats. No particular test guideline was followed, and the study was not performed according to GLP. You have assigned this study a reliability score of 2 (reliable with restrictions). ([redacted] 1965)

You have not provided any study record of a sub-chronic toxicity study (90 day) on the registered substance in the dossier that would meet the information requirement of Annex IX, Section 8.6.2.

In the technical dossier you have provided a study record for two studies on the registered substance (studies 1-2 above). However, these studies do not provide the information required by Annex IX, Section 8.6.2., for the following reasons:

- For study 1: the exposure duration is less than 90 days, and the number of animals examined per dose is significantly lower than in the 90 day sub-chronic toxicity study.
- For study 2: the exposure duration is less than 90 days, and the study does not provide any data on opthalmological examination, haematology, urinalysis, clinical chemistry, or neurobehavioral examinations, and the study does not include any histopathological examination.

In addition to the information on the registered substance, you have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by

providing study records (studies 2-7 above) for two analogue substances 3-methoxybutyl acetate (EC No 224-644-9) (source substance 1) and butane-1,3-diol (EC No 203-529-7) (source substance 2).

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

In addition to the deficiencies pointed out in Appendix 1, section 0 of this decision, ECHA also notes that Annex XI, Section 1.5 provides with regard to the reliability and adequacy of the source studies that in all cases the results of the read-across should:

- *be adequate for the purpose of classification and labelling and/or risk assessment,*
- *have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3),*
- *cover an exposure duration comparable to or longer than the corresponding test method referred to in Article 13(3) if exposure duration is a relevant parameter, and*
- *adequate and reliable documentation of the applied method shall be provided.*

ECHA notes the following observations:

- Study 3: the study does not contain a histopathology of all tissues/organs required as part of a 90 day sub-chronic toxicity study. ECHA notes that only the following tissues were examined histopathologically: testes, ovaries, and pituitary gland. No (neuro)behavioural examination, or ophthalmology was included, organ weights were not measured, and no gross pathology was conducted as part of the study
- Study 4: the exposure duration is less than 90 days, and the number of animals examined per dose is significantly lower than in the 90 day sub-chronic toxicity study.
- Studies 5 and 6: ECHA notes that the following organs/tissues were not examined histopathologically: spinal cord, thymus, oesophagus, salivary glands, trachea, aorta, uterus, accessory sex organs, female mammary gland, prostate, lumph nodes and skin. Furthermore, ECHA notes that the studies do not include any opthalmological examination, clinical biochemistry, or neurobehavioral examination of the animals.
- Study 7: ECHA notes that the study did not include any haematology examinations, and the clinical chemistry included only examination of serum glucose and ketone bodies, whereas the urinalysis examined only urine ketone bodies. The study did not include any histopathological examination, opthalmological examination, or (neuro)behavioural examinations.

ECHA notes that none of the studies provided on either of the analogue substances provide the information required by Annex IX, Section 8.6.2., for the reasons described above.

Therefore, your adaptation of the information requirement is rejected.

In your comments on the draft decision you once again indicate that the above seven studies together with the conclusions from EFSA, WHO & RIFM of other branched chain alcohols and related esters demonstrate that there is no concern for systemic toxicity after repeat-dose exposures at concentrations up to a limit dose of 1000 mg/kg/day for the substances in your read across approach. As already indicated above, under Annex 1, Section 0, of this decision, the studies with the analogue substances 3-methoxybutan-1-ol (EC no 219-741-8) and butane-1,3-diol (EC no 203-529-7) cannot be considered for the assessment of the weight of evidence adaptation as currently the read-across approach

cannot be accepted. Furthermore, as explained above, the studies with the analogue substances have individual shortcomings, hence the studies you have reported, individually or taken together, do not provide the information required by Annex IX, Section 8.6.2. With reference to the two studies with the registered substance, as explained above, the exposure duration in these studies is less than 90 days.

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

ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration. More specifically, even though the information indicates that human exposure to the registered substance by the inhalation route is likely, there is no concern for severe local effects following inhalation exposure. Hence, the test shall be performed by the oral route using the test method OECD TG 408.

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

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

Following a proposal for amendment (PfA), submitted by one of the Member States Competent Authorities, you agreed to perform the OECD TG 408 study with the registered substance. In your comments you indicated that this study (OECD TG 408) could "*further inform on the validity of the read-across*" and on the "*decision regarding the need to conduct an EOGRTS or, if ultimately necessary, the protocol design for performing an EOGRTS*" with the registered substance.

Notes for your consideration

The Extended one-generation reproductive toxicity study (EOGRTS) according to Annex X, Section 8.7.3. is not part of this decision because the results of the Sub-chronic toxicity study (90-day) are considered crucial to inform on the study design of the EOGRTS. Therefore, the results of the Sub-chronic toxicity study (90-day) should be used, among other relevant information, to decide on the study design of the EOGRTS.

ECHA may therefore launch a separate compliance check at a later stage addressing the EOGRTS information requirement.

Alternatively, you may also consider submitting a testing proposal for an Extended one-generation reproductive toxicity study together with the results of the requested Sub-chronic toxicity study (90-day). The testing proposal should include a justification for its study design following ECHA Guidance on information requirements and chemical safety

assessment Chapter R.7a, Section R.7.6 (version 6.0, July 2017), taking into account the results of the Sub-chronic toxicity study (90-day).

2. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* gene mutation study in bacteria" is a standard information requirement as laid down in Annex VII, Section 8.4.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

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

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

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

In your dossier, you have provided the following information for this endpoint:

- An *in vitro* gene mutation study in bacteria, on the registered substance, using OECD TG 471, on the registered substance ([redacted] 1992)

According to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used: S. typhimurium TA1535; TA1537 or TA97a or TA97; TA98; TA100; S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). This includes four strains of S. typhimurium (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four S. typhimurium strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by E.coli WP2 strains or S. typhimurium TA102 which have an AT base pair at the primary reversion site.

You have provided a tests from the year 1992 according to OECD TG 471 and GLP with an assigned reliability score of 1. The test used five different strains of S. typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100 and it did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). However, since the

test was conducted, significant changes have been made to OECD TG guideline 471 so that additionally testing with S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101) is now required. Therefore, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

ECHA concludes that a test using E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102 has not been submitted and that the test using one of these is required to conclude on in vitro gene mutation in bacteria.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing (a) study record(s) for a an in vitro gene mutation study in bacteria, on the analogue substance 3-methoxybuanol (EC No 219-741-8) (source substance 1) ([redacted] 1992).

However, as explained above in Appendix 1, section 0 of this decision, your adaptation of the information requirement is rejected. In addition, ECHA notes that as with the corresponding study on the registered substance, this particular study also did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). Therefore, in additoin to the shortcomings noted in your grouping and read-across approach, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

Therefore, your adaptation of the information requirement is rejected.

In your comments on the draft decision you indicated that a study performed before 1997, that is before the OECD TG 471 was updated, should not trigger a requirement for a new study requesting the additional strain(s), "*unless there is a sound scientific rationale*". Moreover, you stated that the registered substance "*is clearly not an oxidizing compound, cross linking agent, or hydrazine*".

ECHA notes that the fifth strain in the Ames test, as specified in the current test guideline, increases the ability of the test design to detect mutagenicity. It is known that the *S. typhimurium* strains in the old guideline version may not detect certain oxidising mutagens, cross-linking agents and hydrazines. *E. coli* WP2 uvrA or *S. typhimurium* TA102 are sensitive to a variety of oxidative mutagens which are not detected by the standard strains of the Ames test. Since in the technical dossier there is a negative Ames study tested with only the following strains: *S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100, it cannot be concluded on mutagenicity as a whole. Hence, the available study record [redacted], 1992) fails to provide adequate information for the purpose of classification and labelling and/or risk assessment and adequate and reliable coverage of the key parameters foreseen to be investigated in the OECD TG 471 as per REACH Annex XI, Section 1.1.2. (1) and (2).

ECHA notes that the registered substance indeed is not a hydrazine. However, you have not considered the potential cross-linking or oxidising metabolites of the registered substance. Therefore, the use of the *E. coli* WP2 uvrA or *S. typhimurium* TA102 strain is required to determine whether the registered substance is an oxidising mutagen and/or cross-linking agent.

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

ECHA considers that the bacterial reverse mutation test (test method EU B.13/14. / OECD TG 471) is appropriate to address the standard information requirement of Annex VII, Section 8.4.1. of the REACH Regulation.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to complete following information derived with the registered substance subject to the present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102.

3. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing (a) study record(s) for an *in vitro* mammalian chromosome aberration study (OECD TG 473) with the analogue substance 3-methoxybuanol (EC No 219-741-8). Furthermore, you have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing study records for an *in vivo* dominant lethal assay, with an unspecified guideline, and not performed according to GLP (Hess et. Al 1981), and an *in vivo* cytogenitic study, performed with an unspecified guideline, and not according to GLP (Hess et. Al 1981) both performed with the analogue substance butane-1,3-diol (EC No 203-529-7).

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

In your comments on the draft decision you once again indicated that there is an OECD TG 473 study with the analogue substance 3-methoxybutan-1-ol (EC No 219-741-8) and *in vivo* studies with the analogue substance butane-1,3-diol (EC No 203-529-7). However, as already indicated above under Appendix 1, Section 0 of this decision, the study with this analogue substance cannot be considered for the assessment of this standard information requirement, as currently the read-across approach cannot be accepted.

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

ECHA considers that the *in vitro* mammalian chromosome aberration test (test method OECD TG 473) and the *in vitro* mammalian cell micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

4. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained.

ECHA notes that the registration dossier does not contain appropriate study records for this endpoint. Adequate information *on in vitro* gene mutation in mammalian cells will however need to be present in the technical dossier for the registered substance to meet this information requirement provided that both studies requested under 2 and 3 have negative results. ECHA set the deadline for provision of the information to allow for sequential testing.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing (a) study record(s) for an *in vitro* mammalian cell gene mutation study (OECD TG 476) with the analogue substance 3-methoxybutan-1-ol (EC No 219-741-8.

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

In your comments on the draft decision you once again indicated that there is an OECD TG 476 study with the analogue substance 3-methoxybutan-1-ol (EC No 219-741-8). However, as already indicated above under Appendix 1, Section 0 of this decision, the study with this analogue substance cannot be considered for the assessment of this standard information requirement, as currently the read-across approach cannot be accepted.

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

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the

thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that both studies requested under 2. and 3. have negative results.

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

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Pre-natal developmental toxicity studies (test method OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

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

However, there is no information provided for a pre-natal developmental toxicity study in a second species.

ECHA notes that in the technical dossier you also have a pre-natal developmental toxicity study in rats by the oral route with the analogue substance butane-1,3-diol (EC No 203-529-7). However, as explained above in Appendix 1, section 0 of this decision, currently the read-across cannot be accepted. Moreover, ECHA notes that the study is also in rats (first species) hence currently in the dossier there is also no information available on the second species with the analogue substance(s).

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

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

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

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

Following a proposal for amendment (PfA), submitted by one of the Member States Competent Authorities, you stated that the request for the OECD TG 414 study in the second species is not justified, mainly because of the following considerations:

(i.) "*According to Annex IX, section 8.7.2, [...] column 2*" the "*decision on the need to perform a study at this tonnage level or the next on a second species should be based on the outcome of the first test and all other relevant available data*" such as "*new 90-day-study, new toxicokinetic data, empirically supported read-across*";

(ii.) You assert that there is low toxicological activity, and thus, additional studies do not need to be conducted because no hazards will be identified with further animal experimentation.

(iii.) There is a "*continuing scientific and regulatory debate regarding the usefulness of a rabbit developmental toxicity study in identifying effects that are not characterized by rat developmental toxicity studies*"; and

(iv.) Before requesting two species developmental toxicity studies, other information needs to be present such as "*positive genotoxicity data*".

With reference to your first consideration in point (i.) ECHA notes that you are referring to Annex IX, however this substance (3-methoxybutyl acetate) is registered at Annex X. At this tonnage level, according to Annex X, Section 8.7.2, a pre-natal developmental toxicity study conducted on a second species is a standard information requirement.

As regards to point (ii.), ECHA acknowledges that there is a possibility to invoke a column 2 adaptation (Annex X, Section 8.7.) if there is low toxicological activity, *and* no systemic absorption *and* no significant human exposure. These criteria are cumulative. You have not demonstrated that there is no systemic absorption, nor have you demonstrated that there is no significant human exposure (you have not performed an exposure assessment in the Chemical Safety Report). Therefore this adaptation fails.

As regards to point (iii.), ECHA notes that the alleged existence of a debate is not a valid adaptation under column 2 of Annex X, Section 8.7, nor under Annex XI of the REACH Regulation.

Finally, referring to point (iv.), ECHA reinstates that REACH Annex X, Section 8.7.2., is a standard information requirement and is not conditional upon the existence of a positive genotoxicity study. The absence of a positive genotoxicity study is not a valid adaptation under column 2 (Annex X, Section 8.7.) nor under Annex XI of the REACH Regulation.

Appendix 2: Procedural history

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

The compliance check was initiated on 29 August 2017.

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

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

ECHA took into account your comments and did not amend the requests however amended the deadline.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

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

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

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-62 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

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

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.

4. ECHA notes that this case is related to a separate decision CCH-D-2114453554-47-01/F on a compliance check on the source substance (3-methoxybutan-1-ol (EC No 219-741-8)).