EUROPEAN CHEMICALS AGENCY

Helsinki, 12 March 2020

Addressees
Registrants of [redacted] listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision
20/06/2019

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Antimony
EC number: 231-146-5
CAS number: 7440-36-0

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

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***17 June 2021***.

a) Requirements applicable to all the Registrants subject to Annex VII of REACH

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method EU B.13/14. / OECD TG 471) with the Substance

b) Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. 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 Substance

2. Only if both studies under section A.1 and B.1. have negative results, In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method OECD TG 476 or TG 490) with the Substance

Conditions to comply with the requested information

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.
To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annex VII of REACH, if you have registered a substance at 1-10 tonnes per annum (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa;
- you have to comply with the requirements of Annexes VII, VIII and IX of REACH, if

you have registered a substance at 100-1000 tpa;

- you have to comply with the requirements of Annexes VII to X of REACH, if you have registered a substance at above 1000 tpa.

The Appendix on general considerations addresses common arguments that are applicable throughout the present decision while the other Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

The test material used to perform the required studies must be selected and reported in accordance with the specifications prescribed in the Appendix entitled Observations and technical guidance.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

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.

Approved[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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

Appendix on general considerations

The ECHA Guidance documents referred to in this decision are listed in Appendix D of this decision.

In the initial submission, on which the draft decision was based and provided to you for commenting, you did not provide any documentation for your weight-of-evidence and read-across approach.

In your comments and your updated registration you have provided a justification for your weight-of-evidence and read-across approach. You also refer to version 2 of your revised documents entitled "Scientific opinion, weight of evidence and read-across assessment, and further research options Human Health:one to cover Genotoxicity and one for Reproductive Toxicity, both dated 17 June 2019" included in section 13 of your submission on 20 June 2019 .

(i) Assessment of the weight-of-evidence adaptations, in light of the requirements of Annex XI, Section 1.2.

You have adapted the following standard information requirements by applying weight-of-evidence approaches in accordance with Annex XI, Section 1.2:

- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.,
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.;

In the update you have provided the following justification for your weight-of-evidence

You argue that there is sufficient weight of evidence from a grouping and read across approach and from other studies investigating parameters of genotoxicity to show that Sb compounds, have clastogenicity *in vitro.* Also they are not genotoxic *in vivo*.

We assessed the new information you provided and identified the following issue(s):

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

According to ECHA Guidance R.4.4, a weight of evidence adaptation involves an assessment of the relative values/weights of different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance of the information for the given regulatory information requirement. Subsequently, relevance, reliability, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the dangerous property investigated by the required study.

All of the sources of information you have provided are on analogoue substances for which the read-across approach is rejected as explained in Section (ii). Therefore this information does not allow for a reliable conclusion on the dangerous property under investigation.

Specific considerations for the individual endpoints also result in a failure to meet the requirement of Annex XI, Section 1.2. These are set out under the endpoints concerned.

As explained above, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.2. Therefore, your adaptations are rejected.

(ii) Assessment of the Grouping of substances and read-across approach, in light of the requirements of Annex XI, Section 1.5.

You have adapted the following standard information requirements by applying read-across approaches in accordance with Annex XI, Section 1.5:

- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.),
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);

We assessed the new information you provided and identified the following issue(s):

ECHA has considered the scientific and regulatory validity of your grouping and read-across approach in general before assessing the specific standard information requirements in the following appendices.

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. 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 (addressed under 'Scope of the grouping'). 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.

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance R.6 and related documents.

Scope of the grouping

In the justification document you report that you have grouped antimony and antimony compounds. You have further identified three sub-groups according to valency and other parameters such as in vitro gastric bioaccessibility. For sub-group Sb3+ you have used valency of III and bioaccessibility to define the group which comprises the following substances:

Antimony (EC 231-146-5, CAS 7440-36-0)
Diantimony trioxide (ATO,EC 215-175-0, CAS 1309-64-4);
Antimony sulphide (ATS, EC 215-713-4, CAS 1345-04-6);
Antimony trichloride (ATC, EC 233-047-2, CAS 10025-91-9); and
2,5,7,10,11,14-hexaoxa-1,6-distibabicyclo[4.4.4]tetradecane (ATEG, EC 249-820-2, CAS 29736-75-2)

You provide the following reasoning for the grouping of the substances: the substances show limited release of Sb^{3+} ion in bio-elution tests and have moieties or impurities which do not have a greater systemic toxicity profile than Sb^{3+} ion. You consider that the moieties are either essential elements, with none/negligible reproductive toxicity or normal metabolities which are readily metabolized. You exclude substances if there is evidence that the final speciation of released ions is not comparable.

ECHA understands that this is the applicability domain of the Sb^{3+} grouping and will assess your predictions on this basis.

A. Predictions for properties

ECHA understands that you intend to apply a grouping and read across approach as part of your weight of evidence using a read-across hypothesis which is based on the formation of common (bio)transformation products. Namely, that the above grouping are substances which release Sb 3+ ions which may be available for absorption and drive the toxicity profile of the substances. ECHA understands that the properties of your Substance are predicted to be quantitatively equal to those of the source substance. You further consider that the difference in moieties can be omitted for the purposes of read-across.

ECHA notes the following shortcoming(s) with regards to prediction(s) of toxicological properties.

Missing supporting information

Annex XI, Section 1.5 of the REACH Regulation states that "physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)". For this purpose "it is important to provide supporting information to strengthen the rationale for the read-across"[2]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on other category members.

Supporting information may include toxicokinetic information on the formation of the common compound, bridging studies to compare properties of the Substance and source substance.

Missing supporting information on the formation of common compound

As indicated above, your read-across hypothesis is based on the (bio)transformation of the category members to a common compound(s). In this context, information characterising the, rate and extent of the transformation of the category members is necessary to confirm the formation of the proposed common biotransformation product and to assess the impact of the exposure to the parent compounds.

In your justification document, you refer to recent aqueous solubility data and *in vitro* bioelution assays conducted using artificial gastric fluid for your antimony substances ([redacted], 2019).

The bioaccessibility data from the *in vitro* bioelution assays show that for Group Sb3+ it is antimony metal powder which is most soluble and has highest 'oral' bioaccessibility.

ECHA considers that the *in vitro* bioaccessibility data does not provide information on systemic absorption and bioavailability. Therefore, it cannot currently be assessed whether the *in vitro* bioaccessibility results provide the basis for predicting *in vivo* toxicity. Further information would be needed to confirm the relevance of the *in vitro* bioaccessibility results for predicting *in vivo* toxicological properties following the oral route of exposure. Such information to allow comparison between the substances could include information from *in vivo* toxicokinetics and information on the toxicodynamic properties of the substances in your Sb3+ grouping.

[2] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

You have not provided any *in vivo* toxicokinetic data that would confirm your bioaccessibility based approach; therefore it is impossible to translate bioaccessibility into *in vivo* bioavailability which is the parameter of interest for read-across predictions.

Futhermore, you refer to a draft report [redacted], 2017 which indicates that as a generalization uptake efficiency is <1%. However, differences in absorption were observed for some substances in your Sb^{3+} group (for example ATO versus ATC). The authors consider that differences in the solublity and the counter ion of the antimony compound impacts absorption *in vivo*. It has not been established whether this impacts the prediction of properties.

ECHA considers that you have not addressed whether differences in absorption impact your read across hypothesis and that in vivo relevance of the bioaccessibility model can not be confirmed.

Read-across hypothesis contradicted by existing genotoxicity data

Annex XI, Section 1.5. provides that "substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances. The ECHA Guidance[3] indicates that "*it is important to provide supporting information to strengthen the rationale for the read-across*". The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s) in your Sb3+ grouping. The observation of differences in the toxicological properties among some members of a category is a warning sign. An explanation for such a difference resulting in a contradiction between the similarities in properties claimed in the read-across hypothesis and the observation of different properties needs to be provided and supported by scientific evidence.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar target and source substances in your Sb3+ grouping cause the same type of effect(s).

However, the results of the information on mutagenicity obtained with the category members vary. Specifically, positive results are observed in the *in vitro* gene mutation study in bacteria with your Substance while negative results are reported for equivalent studies *in vitro* gene mutation study in bacteria conducted with ATO and ATC which are in your Sb3+ grouping. You have no other information from other tests which would allow you to confirm that the substances have the same mutagenic properties. Although you have available *in vivo* studies performed with ATO you have no information with other analogues in your grouping that would address the same type of mutagenicity. Hence, a comparison of same type of effects is not possible.

The available set of data on the target and source substances in your Sb3+ grouping indicates differences in the toxicological properties of the substances. This contradicts your read-across hypothesis whereby the structurally similar target and source substances in your Sb3+ grouping cause the same type of effect(s). Therefore you have not demonstrated and justified that the properties of the category members are likely to be similar despite the observation of these differences.

[3] Guidance on information requirements and chemical safety assessment (version 6.0, July 2017), Chapter R.6, Section R.6.2.2.1.f

In addition to the general issues with your read-across approach, we have also assessed the reliability of the studies you have provided and identified specific issues. You will find details of these specific issues for each standard information requirement in the following appendices.

Therefore your read-across adaptations are rejected since they do not comply with the rules set out in Annex XI, Section 1.5.

iii. Your comments on your testing programme

In your comments you refer to a testing programme provided also as a matrix. Under this programme you intend to generate information to address the further testing needs you have identified in the justification document and strengthen your read across and weight of evidence approaches. You plan to measure the general mechanisms for genotoxicity in cells to inform on your read-across hypothesis. Once the hypothesis of genotoxicity mechanism of action is refined the ideal Sb substance test item for inhalation tests is identified. Furthermore you intend to verify possible false positive effect of the staining used in the *in vitro* micronucleus assays in order to validate your availablemicronucleus data. You also plan to rank Sb substances as to lowest and greatest oral bioavailability and identify "ideal" substances for further investigation, conduct 2 week oral dose range finder/tolerability studies, conduct oral reproductive/developmental toxicity screening studies (OECD 422) on one or two substances per group and then consider need of any pre-natal developmental toxicity study(ies) (OECD 414).

Concerning the sequential testing ECHA notes that you are planning a series of studies in order to substantiate the read across hypothesis and generate the necessary supporting information and source studies to support your adaptations.

ECHA notes that it is at the discretion of the registrant to undertake additional testing to substantiate your read-across but the outcome of the testing programme may or may not confirm your hypothesis. The timeline in the decision allows for sequential testing of OECD 421/2 and OECD 414 studies and also for OECD 471, 473/487 and conditional 476/490 OECD studies.

Appendix A: Reasons for the requests to comply with Annex VII of REACH

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 1 to 10 tonnes or more per year must contain, as a minimum, the information specified in Annex VII to the REACH Regulation.

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

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

You have adapted the standard information requirement according to Annex XI, Section 1.2. (weight-of-evidence) and Annex XI, Section 1.5. (read-across) of REACH. In support of your adaptation of this information requirement, you have provided the following supporting information for this endpoint:

i. a weight-of-evidence record for an Ames reversal bacterial test, [redacted] 1998 (publication), performed according to OECD TG 471, GLP not specified, with the analogue substance diantimony trioxide - dioxodistiboxane (ATO), purity of test material not specified with the following strains, S. typhimurium TA 1535, TA 1537, TA 98, TA 100, E. coli WP2 uvr A and E. coli WP2 which all gave negative results.
ii. a weight-of-evidence record for a non guideline, non GLP Ames Salmonella test, Kuroda 1991 (publication), with the analogue substance ATO with the following strains, S. typhimurium TA 100 and TA 98 which both gave negative results.
iii. a weight-of-evidence record for a non guideline, non GLP Ames Salmonella test, Kuroda 1991 (publication), with the analogue substance antimony trichloride (ATC) with the following strains, S. typhimurium TA 100 and TA 98 which both gave negative results.
iv. a weight-of-evidence record for a non guideline, non GLP B. subtilits rec assay, Kuroda 1991 (publication), with the analogue substance ATC with the following strain, Bacillus subtilis M45(rec-) and H17(rec+) with a positive test result.
v. a supporting record for non guideline, non GLP DNA damage test in the B. subtilits rec-assay, Kuroda 1991 (publication), with the analogue substance ATO with the following strains, S. typhimurium TA 100 and TA 98 which both gave negative results.
vi. a supporting non guideline, non GLP Bacillus subtilis rec assay, Kanematsu 1980 (publication), with the analogue substance ATO with a positive test result.
vii. a supporting non guideline, non GLP supporting SOS chromotest, Lantzsch 1997 (publication), with the analogue substance ATC using E. coli (PQ37) with a negative result.
viii. a disregarded non guideline, non GLP Reverse mutation assay, Kanematsu 1980 (publication), performed with the analogue substance ATO. Strains: S. typhimurium TA 1535, 1537, 98, 100, 1538 and E. coli WP2 which all gave negative results.
ix. a weight-of-evidence record for a non guideline, non GLP Ames Salmonella test, Asakura 2009 (publication), with the Substance Antimony with the following strains S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 uvr A pKM 101 which had a positive result in strain TA 1537 without metabolic activation, as provided in your updated dossier.

You have not provided a key study conducted with the Substance in your dossier.

For the reasons explained in the Appendix on General considerations regarding weight-of-evidence and read-across, your adaptations are rejected.

In addition to the generic problem of your read-across approach, we have also assessed the reliability of the studies on analogue substances submitted for this standard information, requirement in case you intend to consolidate your read-across adaptation. We have identified the following issues with the studies.

A. More specifically, to fulfil the information requirement, the studies have to meet the requirements of OECD TG 471 (1997)[4]. The key parameter(s) of this test guideline include:

1. The maximum dose tested must induce a reduction in the number of revertant colonies per plate compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test dose must correspond to 5 mg/plate or 5 ml/plate.

   However, in study i. listed above this key parameter was not reported.

2. Triplicate plating must be used at each dose level.

   However, in study i. listed above triplicate plating was not used.

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

   However, in studies ii., iii. and v. the strains TA1535; TA1537 or TA97a or TA97 and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101) was not used. In studies iv., vi. and vii. listed above none of the required strains were used.

4. Two separate test conditions must be assessed: in the absence of metabolic activation and in the presence of metabolic activation.

   However, in study viii. listed above two test conditions were not reported.

5. One positive control must be included in the study. The positive control substance must produce a statistically significant increase in the number of revertant colonies per plate compared with the concurrent negative control.

   However, in study viii. listed above a positive control was not included.

The information provided does not cover the key parameter(s) required by OECD TG 471.

We have assessed the newly provided information from your comments and update and identified the following additional issue(s):

B. According to Article 13(4) of REACH, Ecotox and toxicological tests and analyses must be conducted in compliance with GLP or an equivalent international standard. Annex XI furthermore provides criteria that must be met for non-GLP studies. The Annex XI, section 1.1. refers to "existing data" available at the commencement of the Regulation. Hence, the adaptation only applies to studies conducted prior to 1 June 2008.

You have included a publication for a non-guideline non GLP *in vitro* gene mutation study

[4] ECHA Guidance R.7a, Table R.7.7-2, p.557

in bacterial cells by Asakura et al. from year 2009 (ix.) labelled with reliability 2 performed with the Substance in five strains (S. typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli WP2 uvr A pKM 101 with and without metabolic activation system. The test result was positive in strain TA 1537 without metabolic activation.

ECHA notes that the provided non-guideline non GLP data is from year 2009. Hence, for your study conducted after 1 June 2008 Annex XI, section 1.1. does not apply. Therefore, the study does not fulfil the information requirement.

Based on the above, the information requirement is not fulfilled.

Appendix B: Reasons for the requests to comply with Annex VIII of REACH

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 10 to 100 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII and VIII to the REACH Regulation.

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

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is a standard information requirement in column 1 of Section 8.4.2. of Annex VIII to REACH. Column 2 of this section sets out that this information may be omitted if data from an *in vivo* test is available.

a) *In vitro* information required in Column 1 of Section 8.4.2. of Annex VIII

You have adapted the standard information requirement according to Annex XI, Section 1.2. (weight-of-evidence) and Annex XI, Section 1.5. (read-across) of REACH. In support of your adaptation of this information requirement, you have provided the following supporting *in vitro* information for this endpoint:

i. a weight-of-evidence record for a chromosomal aberration test, [redacted] 1998 (publication), according to OECD TG 473 but no GLP with the analogue substance ATO in human lymphocytes with a positive result.
ii. a weight-of-evidence record for a non guideline non GLP micronucleus study, Gebel 1998 (publication) in V79 cells with the analogue substances ATO and ATC with positive results.
iii. a weight-of-evidence record for a non guideline non GLP micronucleus test, Schaumloffel 1998, with the analogue substance ATC in human lymphoytes with a positive test result.
iv. a weight-of-evidence record for a non guideline non GLP micronucleus test, Huang 1998 (publication) with the analogue substance ATC in CHO cells, human fibroblasts and bronchial epithelial cells with positive results.
v. a supporting SCE test Kuroda 1991 (publication) similar to OECD TG 479, no GLP, with the analogue substance ATO in chinese hamster cells with a positive result.
vi. a disregarded SCE test, Gebel 1997 (publication), performed similarly to OECD TG 479, no GLP with the analogue substance ATO with a positive result.
vii. a weight-of-evidence record for a non reliable non guideline non GLP chromosomal aberration test, Asakura, 2009 with the Substance Antimony in Chinese hamster lung cells with a positive result, (as provided in your updated dossier).

You have not provided a key study conducted with the Substance in your dossier.

For the reasons explained in the Appendix on General considerations regarding weight-of-evidence and read-across, your adaptations are rejected.

In addition to the generic problem of your read-across approach, we have also assessed the reliability of the studies on analogue substances submitted for this standard information, requirement in case you intend to consolidate your read-across adaptation. We have identified the following issues with the studies.

A. To fulfil the information requirement, the study has to be an *in vitro* chromosomal aberration test or an *in vitro* micronucleus test, conducted in mammalian cells in

accordance with OECD TG 473 or OECD TG 487, respectively[5]. The key parameters of these test guidelines include:

1. At least 300 well-spread metaphases must be scored per concentration (OECD TG 473).

   In study i. listed above only 100 metaphases were scored.

2. At least 2000 cells must be scored per concentration (OECD TG 487).

   In studies ii. to iv. listed above only 1000 cells were scored.

B. To fulfil the information requirement, the study has to be an *in vitro* chromosomal aberration test or an *in vitro* micronucleus test, conducted in mammalian cells in accordance with OECD TG 473 or OECD TG 487[6].

   However, studies v. and vi. are neither an *in vitro* cytogenicity study in mammalian cells nor an *in vitro* micronucleus study.

We have assessed the newly provided information from your comments and update and identified the following additional issue(s):

C. According to Article 13(4) of REACH, Ecotox and toxicological tests and analyses must be conducted in compliance with GLP or an equivalent international standard. Annex XI furthermore provides criteria that must be met for non-GLP studies. The Annex XI, section 1.1. refers to "existing data" available at the commencement of the Regulation. Hence, this adaptation only applies to studies conducted prior to 1 June 2008.

   You have included a publication for a non-guideline non GLP *in vitro* chromosomal aberration study (Asakura, 2009) labelled as reliability 3 (not reliable and significant methodological deficiencies) (vii.) performed with the Substance in Chinese hamster lung cells with and without metabolic activation system. The test result was positive. You did not include this study in your formal comments on the draft decision.

   ECHA however notes that the provided not reliable non-guideline non GLP study is from year 2009. Hence, for your study conducted after 1 June 2008 Annex XI, section 1.1. does not apply. Therefore, the study does not fulfil the information requirement.

Therefore, the information provided does not cover key parameter(s) required by the relevant OECD TG.

b) *In vivo* information justifying an adaptation under Column 2 of Section 8.4.2. of Annex VIII

Annex VIII, section 8.4.2, column 2 sets out that an *in vitro* cytogenicity study does not need to be conducted if adequate data from an *in vivo* cytogenicity test are available.

While you have not provided data from an *in vivo* cytogenicity test with the Substance, you have provided *in vivo* information on analogue substances in support of your adaptation for this endpoint. We understand that you rely on Annex XI, Section 1.2. (weight-of-evidence) and Annex XI, Section 1.5. (read-across) of REACH to replace the *in vivo* cytogenicity test

[5] ECHA Guidance R.7a, Table R.7.7-2, p.557
[6] ECHA Guidance R.7a, Table R.7.7-2, p.557

required to benefit from the adaptation under Annex VIII, section 8.4.2, column 2. You provided the following:

- *In vivo* chromosomal aberration studies:

  i. a supporting repeated dose (21 days) exposure chromosomal aberration study, [redacted] 2005/2006 (study report), performed according to OECD TG 475 and GLP with the analogue substance ATO in rat via oral (gavage) route with a negative result.
  ii. a weight-of-evidence record for a mammalian spermatogonial CA test, [redacted] 2007 (publication) according to OECD TG 483 and GLP with the analogue substance ATO in rat via oral (gavage) route with a negative result.
  iii. a disregarded chromosomal aberration test with single acute exposure, [redacted] 1992, performed similarly to OECD TG 475 with deviations, no GLP specified, with the analogue substance ATO in male mouse via oral (gavage) route with an ambiguous result.
  iv. a disregarded chromosomal aberration test with repeated exposure, [redacted] 1993 (publication), performed similarly to OECD TG 475, GLP not specified with the analogue ATO in mouse via the oral (gavage) route with an ambiguous result.

- *In vivo* micronucleus studies:

  i. a supporting repeated exposure micronucleus study (21 days), [redacted] 2005/2006 according to OECD TG 474 and GLP with the analogue ATO in rat via oral (gavage) route with 1000 cells scored per animal with a negative result.
  ii. a weight-of-evidence record for a single dose micronucleus test and a supporting repeated dose (21 d) study, [redacted] 1998 (publication), performed according to TG 474, no GLP indicated, in mouse via oral (gavage) route with the analogue substance ATO with 2000 PCEs examined for micronuclei per animal with a negative result.
  iii. a one year [redacted] 2017 erythrocyte micronucleus non guideline study, GLP compliant, in mouse and rat, with the analogue ATO via inhalation route. A positive equivocal result in mouse and a negative result in the rat.
  iv. a weight-of-evidence record for a repeated dose bone marrow micronucleus test, [redacted] 2007 (publication), with the analogue substance ATO according to OECD TG 483 (spermatogonial CA test) and GLP in rat (male/female) via the oral route (gavage) with a negative result.
  v. a weight-of-evidence record for a non guideline non GLP micronucleus test in hamster (sex and strain not specified), [redacted] 1998 (publication), route of administration not specified) with the analogue substance antimony trichloride (ATC) with a positive test result.

- *In vivo* comet study:

  i. a disregarded non test guideline one year comet assay test, [redacted] report, 2017, GLP compliant, in rat and mouse (male/female) with the analogue substance ATO via inhalation route (analysed tissues not indicated) with a positive equivocal result in rat.

- *In vivo* SCE study:

  i. a non guideline non GLP SCE test Gebel 1997 (publication), with the analogue substance ATC in human lymphocytes with a weak positive result.

You have not provide a key study conducted with the Substance in your dossier.

For the reasons explained in the Appendix on General considerations regarding weight-of-evidence and read-across, your adaptations are rejected.

In addition to the generic problem of your read-across approach, we have also assessed the reliability of the studies on analogue substances submitted for this standard information, requirement in case you intend to consolidate your read-across adaptation. We have assessed this information and identified the following issues:

A. To fulfil the adaptation, under Annex VIII, section 8.4.2, column 2 the study must qualify as "*adequate data from an in vivo cytogenicity test*".

   However, the *in vivo* comet study and *in vivo* SCE study are not *in vivo* cytogenicity tests and so cannot be used to fulfil this adaptation.

B. To fulfil the adaptation, under Annex VIII, section 8.4.2, column 2 the study must qualify as "*adequate data from an in vivo cytogenicity test*". The *in vivo* study must be either a micronucleus test or a chromosomal aberration test, performed according to OECD TG 474 or 475[7]. The key parameters of these test guidelines include:

   a) The highest dose studied must be the maximum tolerated dose (MTD), i.e. the highest dose that is tolerated without evidence of toxicity (e.g. body weight depression or hematopoietic system cytotoxicity, but not death or evidence of pain, suffering or distress necessitating humane euthanasia). The highest dose can also be a dose that produces toxicity in the bone marrow (e.g. a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood).

      However, in the *in vivo* chromosomal aberration and *in vivo* micronucleus studies listed above this key parameter was not reported.

   b) It is not appropriate to perform the test if there is evidence that the test substance, or a relevant metabolite, will not reach the target tissue.

      However, in studies *in vivo* chromosomal aberration and in vivo micronucleus listed above it has not been demonstrated that target tissue exposure to the test substance has occured.

   c) At least 200 metaphases must be analysed for each animal for structural chromosomal aberrations including and excluding gaps (OECD TG 475).

      However, in studies i., iii. and iv. listed above for *in vivo* chromosomal aberrations only 100 metaphases were analysed this key parameter was not met.

   d) The proportion of immature among total (immature + mature) erythrocytes must be determined for each animal (by counting a total of at least 500 erythrocytes for bone marrow and 2000 erythrocytes for peripheral blood) (OECD TG 474).

      However, in studies ii., iii., iv. and v. listed above for *in vivo* micronucleus studies you did not count a total of at least 500 erythrocytes for bone marrow and 2000

[7] ECHA Guidance R.7a, Table R.7.7-3, p.558

erythrocytes for peripheral blood.

e) At least 4000 immature erythrocytes per animal must be scored for the incidence of micronucleated immature erythrocytes (OECD TG 474).

However, in studies i., ii., iii. and v. listed above for *in vivo* micronucleus studies listed above only 1000 cells were score for micronuclei.

f) The proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes must be reported for each group of animals (OECD TG 474).

However, in studies i., ii., iii. and v. listed above for *in vivo* micronucleus studies listed above you did not report this proportion for each group of animals.
Considering both the general deficiencies of your adaptation (as explained under section General considerations) and the specific deficiencies detailed above, your adaptation is rejected.

Therefore, the information requirement is not fulfilled.

2. Only if both studies under sections A.1. and B.1. have negative results In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.);

An *in vitro* gene mutation study in mammalian cells is a standard information requirement in Annex VIII to REACH in case of a negative result in the *in vitro* gene mutation test in bacteria and the *in vitro* cytogenicity test.

Your dossier contains an adaptation for an *in vitro* gene mutation study in bacteria, and an adaptation for an *in vitro* cytogenicity study in mammalian cells or in vitro micronucleus study.

The information for the *in vitro* gene mutation study in bacteria and for the *in vitro* cytogenicity study in mammalian cells or in vitro micronucleus study provided in the dossier are rejected for the reasons provided in sections A.1 and B.1 in Appendices A and B.

The result of the requests for information in sections A.1 and B.1 in Appendices A and B will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3 is triggered.

Annex VIII, section 8.4.3, column 2 sets out an *in vitro* gene mutation in mammalian cells study does not need to be conducted if adequate data from an *in vivo* gene mutation test are available.

You have provided the following *in vitro* and *in vivo* information on analogue substances in support of your adaptation for this endpoint. We understand that you rely on Annex XI, Section 1.2. (weight-of-evidence) and Annex XI, Section 1.5. (read-across) of REACH to benefit from the adaptation under Annex VIII, section 8.4.3, column 2.

(i) a weight-of-evidence record study performed according to OECD TG 476, no GLP with the analogue substance diantimony trioxide (ATO), [redacted] 1998 (publication) in mouse L5178Y lymphoma cells with a negative result.
(ii) an *in vivo* UDS study performed according to OECD TG 486, GLP not specified, [redacted] 1998 (publication) with the analogue substance ATO in rat, single dose, post-

exposure: at 2 or 16 hours after administration hepatocytes were isolated with an negative result.

As explained under section General considerations, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.2. and 1.5. Hence your adaptation is rejected.

In addition to the generic problem of your read-across approach, we have also assessed the reliability of the studies on analogue substances submitted for this standard information, requirement in case you intend to consolidate your read-across adaptation. We have identified the following issues:

A. Tests on substances must be conducted in accordance with the OECD test guidelines or another recognised international test method (Article 13(3) of REACH). To be considered adequate, the *in vitro* study has to meet the requirements of OECD TG 476 or OECD TG 490[8], and more specifically:

   1. The maximum concentration tested must induce 80-90% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration must correspond to 10 mM, 2 mg/mL or 2 µl/mL, whichever is the lowest.

      In the *in vitro* study listed above this key parameter was not reported.

   2. The response for the concurrent negative control must be inside the historical control range of the laboratory.

   In the *in vitro* study listed above it is not demonstrated that the concurrent negative control is outside the historical control range.

   Therefore this study does not provide a reliable coverage of the key parameter foreseen to be investigated in an OECD TG 476 or OECD TG 490 study.

B. To fulfil the adaptation, under Annex VIII, section 8.4.3, column 2 the study must qualify as "*in vivo* mammalian gene mutation test". The *in vivo* study must be a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay, performed according to OECD TG 488[9].

   The *in vivo* UDS test is not a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay. Therefore, your adaptation is rejected.

Considering both the general deficiencies of your adaptation (as explained under section General considerations) and the specific deficiencies detailed above, your adaptation is rejected.

Therefore the information provided does not fulfil the information requirement.

Consequently, you are required to provide information for this endpoint, if the *in vitro* gene mutation study in bacteria and the *in vitro* cytogenicity study in mammalian cells or an *in*

[8] ECHA Guidance R.7a, Table R.7.7-2, p.557
[9] ECHA Guidance Chapter R.7a, Section R.7.7.6.3

vitro micronucleus study provide a negative result.

Information on the study design

To fulfil the information requirement for the Substance, both the *in vitro* mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) and the thymidine kinase gene (OECD TG 490) are considered suitable.

Metal ions can lead to large DNA deletions (ECHA Guidance R7.a). OECD TG 490 is able to detect large DNA deletions. According to OECD TG 476, either the hprt or xprt gene can be used, referred to as HPRT and XPRT test and only the XPRT test can detect large DNA deletions. Thus, if OECD TG 476 is chosen to be performed, the XPRT test shall be conducted.

Appendix C: Procedural history

The compliance check was initiated on 22 January 2019.

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

Among the comments you provided, some comments were of generic nature, i.e. "*Parallel Compliance Check and Substance Evaluation processes*" and "*commitment to minimization of (vertebrate) animals testing*". These comments did not refer to the requests in the decision or to their justifications, but to other general considerations. Accordingly, ECHA explained in a separate communication how they were taken into account.

You were notified in the draft decision that ECHA does not take into account any dossier updates after the draft decision was sent on 18 April 2019. You updated your registration on 20 June 2019. Given the exceptional circumstances, ECHA has taken into account the above dossier update when processing this decision and assessed the revised justification documents and the additional study records. ECHA did not amend the request(s).

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix D: Observations and technical guidance

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

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 the Member States.

4. Test guidelines, GLP requirements and reporting

Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.

Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses shall be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries[10]'.

5. Test material

Selection of the test material(s)

The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

While selecting the test material you must take into account the impact of each constituent/impurity is known to have or could have on the test results for the endpoint to be assessed. For example, if a constituent/impurity of the Substance is known to have an impact on (eco)toxicity, the selected test material must contain that constituent/ impurity.

Technical reporting of the test material

The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values [and other parameters relevant for the property to be tested, in this case crystal structure/phase and particle size distribution. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

[10] https://echa.europa.eu/practical-guides

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers" on the ECHA website (https://echa.europa.eu/manuals).

6. List of references for the Guidance documents[11] referred to in this decision

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 in this decision.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)12

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

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

Appendix E: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fufilled
[redacted]	[redacted]	[redacted]