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Helsinki, 12 March 2020

Registered substance subject to this decision, hereafter 'the Substance': Diantimony trioxide
EC number: 215-175-0
CAS number: 1309-64-4
Date of latest submission(s) considered[1]: 20 June 2019
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressee(s): Registrant(s)[2] of Diantimony trioxide (ATO)

## DECISION ON SUBSTANCE EVALUATION

In accordance with Article 46(1) of the REACH Regulation (Regulation (EC) No 1907/2006), you must submit the following information on the Substance:

**Human Health**

1. Combined *in vivo* mammalian alkaline comet assay (test method: OECD TG 489) with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in mice, inhalation route (nose only). The comet assay must be performed on the following tissues: nasal mucosa, alveolar epithelium, bronchoalveolar lavage cells, and liver parenchyma with and without additional specific investigation on oxidative DNA damage for each of the tissues mentioned. You may perform the two studies separately, with the specifications as above, if the conduct of combined studies is not feasible or would not result in a reduced number of animals used. In such circumstances justification must be provided.

You must provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the chemical safety report by **20 June 2022**.

In addition to the robust study summaries, you must submit the full study report by the same deadline, by attaching it to the relevant endpoint study record in IUCLID.

The deadline for provision of the requested data takes into account the time that you may need to agree on which of the registrant(s) will perform the required tests (3 months is allocated for this) and include the time required for developing an analytical method, conduct of the study, preparation of the study report and reporting in IUCLID.

[1] This decision is based on the registration dossier(s) on the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

[2] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

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The reasons of this decision and any further test specifications of the requirements are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

**Appeal**

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

**For your information**

Overall, there is evidence that after exposure to antimony containing substances an unidentified antimony species (e.g. Sb3+, Sb5+, or methylated Sb) becomes systemically available and causes effects independently of the route of exposure. Therefore, this substance evaluation is conducted in parallel to evaluations for antimony metal (Sb metal, EC 231-146-5, CAS 7440-36-0), 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) as consequently similar initial concerns need to be clarified. For all cases, including yours, a compliance check has been initiated in parallel to assess whether standard information is missing.

Authorised[3] by Christel Schilliger-Musset, Director of Hazard Assessment

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

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**Appendix 1: Reasons**

Based on the evaluation of all relevant information submitted on diantimony trioxide (ATO)[4] and other relevant available information, ECHA concludes that further information is required to enable the evaluating Member State competent authority (MSCA) to complete the evaluation of whether the Substance constitutes a risk to human health.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested to clarify any remaining concerns in the follow up process.

The identification of a potential risk is based on a combination of exposure and hazard information.

According to information in the registration dossiers the Substance is used at industrial sites in the production of:

- flame retarded textiles,
- glass, enamels, functional ceramics and semi-conductors,
- pigments, paints, coatings, ceramics, brake pads,
- fine chemicals,
- in the use as a flame retardant, as a catalyst in the production and use of polyethylene terephthalate (PET films/fibres, resin), of flame retarded textiles, in the plastics and rubber industry, in wood adhesives and in the manufacture of general electrical products.

Professional uses of ATO are uses of:

- diantimony trioxide preparations,
- flame retarded flexible sealing materials,
- ATO containing paints, and
- ATO containing articles.

Therefore, significant exposure of workers and consumers cannot be excluded.

In your comments on the draft decision, you indicate that you *"disagree with this assumption/claim"*. You further elaborate that this is not justified, as in the CSRs "low/controlled exposures have been demonstrated for all these substances" and *"that the exposure of workers to Sb trioxide is well controlled below the DNEL and can therefore not be claimed to be significant"*.

ECHA notes that there is evidence of exposure of humans, as illustrated above, to a Substance that may be a genotoxic carcinogen. The risk assessment you present in the CSR is irrelevant for non-threshold effects as addressed in this decision.

ECHA emphasises that there is exposure and expects that the information requested by this decision will make possible the assessment of the significance of it.
You further declare that ATO "was always transformed during the process into another substance", which was tightly bound into the matrix. Therefore, you would not expect consumer exposure. However, you failed to provide evidence to support your statement, which remains thus speculative.

[4] The registered substance diantimony trioxide will be named ATO in the following text

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Overall description of the concern

Based on several studies cited in the dossier, there is evidence that ATO is carcinogenic (Groth et al., 1986; NTP, 2017; Watt, 1983) and ATO has a harmonised classification as Carc. 2 (H351). The outcome of existing genotoxicity tests is overall inconclusive. However, the electrophilic properties of ATO as well as the fact that there are *in vitro* and *in vivo* genotoxicity tests with a positive outcome indicate that genotoxicity is a possible mechanism for carcinogenic effects. Therefore, a clarification of this aspect is warranted.

Occupational and consumer exposure via oral, dermal, and inhalation route is evident based on the widespread use of ATO as a catalyst in the manufacturing of polymers as well as synergist for flame retardants and catalyst in the production of PET/PES polymers. ATO is used in a variety of industrial and professional settings for the production and use of the Substance itself or in articles. ATO is used e.g. for production of polymers, plastics, and rubber articles; it is used in flame retardant formulations, and products with ATO containing flame retardants (textiles, sealing materials, wood, plastics, and rubber articles). ATO is also used for preparation and use of glass, enamels, semi-conductors, ceramics, pigments, paints, coatings, brake pads, and wood adhesives. Particularly high occupational exposures can be expected in situations where ATO is handled openly, e.g. during transfer operations, during blending in batch processes and during manipulation of pure ATO or ATO bound in materials and articles, and for cleaning and maintenance activities. Exposure is observed for several involved PROCs e.g. PROC 8a, 10, 11 13, 14, 19, 23, 24, 25, and 28.

Explanation of the testing strategy

In your comments on the draft decision, you request awaiting the outcome of ECHA's compliance check and the assessment of the information submitted subsequently before further information is requested from you under substance evaluation.

ECHA considers that there is no need to postpone substance evaluation as the concerns that lead to further information requests in this decision are already established. Specifically for this case, substance evaluation aims at addressing the concern for *in vivo* genotoxicity in site-of-contact tissue after inhalation exposure. The tests requested under compliance check do not resolve this concern.

There is a concern that the carcinogenicity observed in inhalation studies may be linked to genotoxic effects, which could have an impact on the current classification. Some of the observed carcinoma may be caused by a systemically available antimony species and thus be independent of the route of exposure. This needs to be followed up by targeted investigation of the genotoxicity of the Substance subject to this decision in an organ, which may be affected by systemic availability of such a toxophore.

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**1. Combined *in vivo* mammalian alkaline comet assay (test method: OECD TG 489) with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in mice, inhalation route (nose-only). The comet assay must be performed on the following tissues: nasal mucosa, alveolar epithelium, bronchoalveolar lavage cells, and liver parenchyma with and without additional specific investigation on oxidative DNA damage for each of the tissues mentioned. You may perform the two studies separately, with the specifications as above, if the conduct of combined studies is not feasible or would not result in a reduced number of animals used. In such circumstances justification must be provided.**

The concern(s) identified

The substance evaluation identified specific human health concerns with regard to genotoxic effects of Sb compounds in general and ATO specifically. Amongst other Sb-containing compounds ATO has been tested positive in bacteria (ATO and ATC positive in rec assay (Kanematsu et al., 1980; Kuroda et al., 1991)), Sb metal positive in Ames assay (Asakura et al., 2009)) as well as in cell cultures *in vitro* as described below. Additionally, positive and negative *in vivo* studies are available. The existing information raised thus a concern on potential genotoxicity of ATO *in vivo* that need to be clarified with further information.

Why new information is needed

Chromosome mutations have been observed in several *in vitro* studies with ATO. *In vitro* chromosomal aberration tests according to or similar to OECD TG 473 were applied in four studies with different cell lines (human leukocytes, Chinese hamster lung cells, Chinese hamster ovary cells) to assess the mutagenic potential of ATO (Elliott et al., 1998). The study was positive and detected a dose-dependent increase of aberrant cells. The *in vitro* sister chromatid exchange (SCE) test, similar to OECD TG 479, shows positive effects in V79 Chinese hamster cells and human lymphocytes with a dose-dependent increase of SCEs for ATO (Gebel et al., 1997).

*In vivo*, ATO has been tested negative for chromosomal aberrations (OECD TG 475) and micronucleus formation in bone marrow (OECD TG 474) of rats and mice (Elliott et al., 1998; Kirkland et al., 2007). Additionally, ATO has been assessed using the comet assay (OECD TG 489) with lung and blood cells after 12 months whole body inhalation exposure (NTP, 2017). This test was negative for rats for both lung and blood cells and negative for blood cells of mice. For lung cells in exposed mice a significant and dose-dependent increase of percent tail DNA has been observed.

However, the comet assay of the NTP study (2017) is considered as not reliable for several reasons: i) no positive control has been performed, ii) no historical negative/positive control data are presented and iii) no cytotoxicity measurements have been performed. In particular, the lack of cytotoxicity data makes interpretation of positive comet assay data difficult because target tissue toxicity (cytotoxicity) may also result in increased DNA migration (Burlinson et al., 2007; OECD, 2016) and thus, information on cytotoxicity is required in order to assess the biological relevance of a positive result (OECD, 2016). Additionally, inflammation (as observed in lung tissue in 100% of animals in all treatment groups during the NTP study) has been associated with increases in DNA migration (OECD, 2016). Thus, interpretation of positive results is unclear. Despite the fact that this study

is not considered reliable, it is plausible that trivalent Sb compounds cause DNA single strand breaks in site-of-contact tissues, like in lung cells after inhalation due to electrophilic properties of trivalent Sb compounds.

In the same study (NTP, 2017), mild but significant and dose-dependent increases in micronucleated erythrocytes in male and female mice following exposure for 12 months by inhalation have been observed (negative in rats) indicating the formation of systemically available genotoxic Sb species. You argue that positive results in micronucleus assays might be a consequence of artefacts caused by inclusion body formation, which can be mistaken for micronuclei if non-DNA specific staining techniques are used. This has been demonstrated in urothelial cells after *in vivo* exposure to arsenic (Cohen et al., 2013; Wedel et al., 2013). There is currently no evidence that positive micronucleus assays in *in vi*tro and *in vivo* assays after treatment with Sb compounds can be a result of artefact formation. However, the possibility of a false positive effect can also not be ruled out on basis of the available data. Additionally, i) no positive control has been performed and ii) no historical negative/positive control data are presented.

In humans, Cavallo et al. (2002) examined peripheral lymphocytes from males occupationally exposed to ATO via inhalation for the presence of micronuclei, sister chromatid exchange, and induction of DNA single strand breaks with the Fpg (formamido-pyrimidine-glycosylase) enzyme-modified comet assay. Significantly increased DNA strand breaks after enzyme treatment compared to the assay without enzyme treatment indicated significantly elevated levels of oxidative DNA damage. A causal relationship between ATO exposure and oxidative DNA damage cannot be established with certainty because co-exposure to other potentially genotoxic substances cannot be excluded.

In another human study, El Shanawany et al. (2017) investigated the DNA damage occurring among Egyptian workers occupationally exposed to ATO. In this study, the positive correlation between urinary Sb levels and DNA lesions (apurinic/apyrimidinic (AP) or abasic site) indicate that Sb might have genotoxic properties in humans. Again, a causal relationship cannot be established with certainty because co-exposure to other potentially genotoxic substances cannot be excluded.

For the endpoint genotoxicity it is stated in your provisional read across justification that available data on Sb substances reveal a general trend of no (*in vivo*) genotoxicity and that *in vitro* genotoxicity observed can be the result of indirect mechanisms, such as oxidative stress ([redacted], 2018). Further, it is stated in the provisional read across justification that most *in vitro* studies yield positive genotoxicity results for clastogenic events but not point mutations and that all high quality *in vivo* studies yield negative genotoxicity results.

Your hypothesis is that trivalent Sb compounds share a common mechanism and induce genotoxic effects indirectly, *i.e.* via oxidative stress or impairment of DNA repair processes, based on commonly observed oxidative stress induction for the five Sb compounds under evaluation (ATO, Sb metal, ATS, ATEG, ATC) (Derr et al., 2017; Hendriks, 2017; Jiang et al., 2016; Zhao et al., 2017) as well as impaired DNA repair for ATC (Grosskopf et al., 2010; Koch et al., 2017). However, there is currently no factual evidence from the available studies clarifying that mechanism. This could for example be the identification of a common systemically available antimony species and the correlation of its presence with observed similar systemic effects. Therefore, the evidence is neither sufficient to allow this conclusion about the mode of action of ATO nor to predict presence or absence of genotoxicity for the purpose of classification and labelling or risk assessment of the Substance.

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In the ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a (ECHA, 2017) it is noted, that highly electrophilic substances with positive results *in vitro* may react with proteins and water *in vivo* and hence be rendered inactive very quickly when distributed in the body and thus producing negative effects in most standard *in vivo* assays, in particular when systemic absorption is required to reach the target cells.

However, such electrophilic substances may be able to express their genotoxic potential at the initial site-of-contact with the body. Consequently, the use of test systems, which detect effects in site-of-contact tissues, is important for a comprehensive risk assessment, *i.e.* a comet assay with lung cells. A significant and dose-dependent decrease of glutathione levels (Snawder et al., 1999; Tirmenstein et al., 1995; Tirmenstein et al., 1997; Wyllie and Fairlamb, 2006), reactivity with proteins (Verdugo et al., 2017), and significant reduction of protein thiols (Harvey, 1971; Tirmenstein et al., 1997) demonstrate the electrophilic properties of trivalent Sb compounds and the capacity to damage cellular structures. This is supported by results of the ToxTracker for Sb metal, ATO, ATS, ATC and ATEG (positive for both, oxidative stress and protein reactivity (Derr et al., 2017; [redacted], 2017)).

The electrophilic properties could explain positive *in vitro* results, positive *in vivo* results in the comet assay with lung cells after inhalation exposure in the NTP study (NTP, 2017) and negative *in vivo* results in the micronucleus and comet assay with bone marrow because the latter requires systemic absorption. Thus, the *in vivo* comet assay with cells from site-of-contact tissue in mice should be repeated to be able to draw a robust conclusion as mice seem to be more sensitive than rats (NTP, 2017). Due to the aforementioned positive micronucleus results in the NTP study (2017), this assay should be combined with an *in vivo* micronucleus assay with a DNA-specific staining technique to exclude potential artefacts via inclusion body formation, which can be mistaken for micronuclei (Wedel et al., 2013) as emphasised by the registrant(s) in the dossier. To additionally investigate the aforementioned genotoxic effects observed in human exposure studies (Cavallo et al., 2002; El Shanawany et al., 2017), the *in vivo* comet assay should be combined with the Fpg (formamido-pyrimidine-glycosylase) or EndoIII (endonuclease III) enzyme-modified comet assay in site-of-contact tissue.

What is the possible regulatory outcome?

Taking into account positive *in vitro* mutagenicity tests and the equivocal outcome of *in vivo* genotoxicity assays as discussed above, together with the high tonnage (>10,000 tpa) and the uses of the Substance, a risk for human health cannot be excluded. The genotoxic potential of the Substance needs to be clarified.

A positive result may be decisive for the classification of ATO as mutagen.

Identification as a local acting soma cell mutagen may also have implications for the assessment of carcinogenic effects and their potential modes of action, possibly resulting in a more stringent classification of carcinogenicity as Carc. Cat. 1B.

Thus, results of the requested study are necessary for an adequate hazard assessment and to conclude on potential risks for workers, consumers, and the general population due to exposure to ATO.

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Considerations on the test method and testing strategy

The *in vivo* comet assay according to OECD TG 489 is deemed the best suitable test method to investigate potential genotoxic effects in site-of-contact tissue. This assay must be performed with mice because mice seem to be more sensitive than rats (NTP, 2017).

ECHA considers that the oral route is usually the most appropriate route of administration for substances except gases for testing for mutagenicity. ECHA notes that the Substance is not a gas. However, inhalation exposure of ATO particles is likely and lung carcinogenic effects have been observed before as well as inconclusive effects in the *in vivo* comet assay and micronucleus assay after inhalation exposure. Thus, ECHA considers that the study must be performed by the inhalation route. In order to avoid a potentially confounding effect from ingested test material as a result of animals grooming themselves the inhalation exposure should be performed nose-only. Nasal mucosa cells together with alveolar epithelial cells and bronchoalveolar lavage cells must be assessed to investigate site-of-contact genotoxicity. As systemic target tissue, liver parenchyma must be investigated in addition to the aforementioned site-of-contact tissues.

For all tissues, the standard alkaline comet assay must be performed with and without Fpg (formamido-pyrimidine-glycosylase) or endonuclease III (EndoIII) enzyme-modification to also assess DNA strand breaks resulting from oxidized DNA bases (8-OHdG) (Cavallo et al., 2002; Collins et al., 1996). The modification of the protocol consists of additional slides to be used in the standard alkaline comet assay (OECD TG 489) which will be treated with enzyme (EndoIII or Fpg) between the lysis and alkaline treatment. It is recommended to use the protocol based on the publication from Ersson et al. (2013) or Collins (2000), as described in detail here:
http://cometassay.com/COmet%20Assay%20with%20DNA%20repair%20enzymes.pdf.

Also, a mammalian erythrocyte micronucleus test according to OECD TG 474 is required, to assess systemic genotoxic effects in the mouse by use of peripheral blood erythrocytes and a DNA specific staining technique. The use of a DNA specific staining technique in the micronucleus test was also suggested by you in the provisional read-across justification in order to assess potential staining artefacts ([redacted], 2018).

The studies OECD TG 489 and OECD TG 474 must be combined and it is the responsibility of you, the Registrant, to ensure that the combination of the requested OECD TG 489 and OECD TG 474 studies does not impair the validity and the results of the information of each individual study.

In case you can justify that the conduct of combined studies is not feasible or would not result in a reduced number of animals used the studies may be conducted separately.

With regard to occupational health and safety concerns when performing inhalation studies involving genotoxic positive controls, positive controls can be administered via intratracheal instillation by the commissioned laboratory. In any case, exposure to ATO must be via inhalation (nose only).

You must submit the full study report for the requested information in the dossier update. Considering the complexity of the case as described above including the combination with non-standard modifications, the provision of a complete rationale of test design and interpretation of results and access to all information available in the full study report (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.) are required to enable the evaluating MSCA to fully

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assess the provided information, including the statistical analysis, and to efficiently clarify the concern for mutagenic effects.

Consideration of alternative approaches

Due to the electrophilic properties of ATO and other trivalent Sb compounds, a particular concern for the Substance subject to this decision is potential genotoxic effects in site-of-contact tissue as outlined above. The *in vivo* comet assay according to OECD TG 489 is deemed the best suitable as well as internationally validated and accepted test method to investigate this concern.

*In vitro* 3D tissue models might pose potential alternatives in future because they offer *in vivo*-like behaviour in terms of viability, proliferation, differentiation, morphology, as well as gene and protein expression. However, ECHA emphasises that these *in vitro* models are not yet validated for particle induced lung-toxic/genotoxic effects and do not examine the complete range of bronchiolar-alveolar compartments and the interaction of all involved cells. Thus, only *in vivo* data resulting from a validated test system will suffice to provide information for a comprehensive hazard assessment.

In addition, the *in vivo* Comet assay can be combined with other tests, such as the requested enzyme modification and the *in vivo* micronucleus test (OECD TG 474), in order to optimise the information output to maximise the predictive power of this study. This will help to gather more mechanistic information and to investigate observations made in human exposure studies.

Thus, this study request is suitable and necessary to obtain information that will allow clarifying whether there is a potential risk for genotoxic effects. More explicitly, there is no equally suitable alternative way available of obtaining this information. ECHA notes that there is currently no method available that could generate the necessary information without the use of vertebrate animals.

Consideration of your comments of draft decision

In your comments on the draft decision you questioned the requested combined method and noted "*that a number of advanced genotoxicity assays of potential relevance to Sb are currently under development, and would provide more precise and accurate results for a wider range of mechanistic events potentially relevant to antimony trioxide animal carcinogenesis*". However, you have not provided any details nor relevant references in this regard. The merits of your comments remain thus unclear and your proposal is not a suitable alternative to the requested information. In contrast, the MNT (OECD TG 474) and the Comet assay (OECD TG 489) are well-described, established and validated test methods. Additionally, the inconclusive inhalation *in vivo* comet and micronucleus assays (NTP 2017) are driving the concern for genotoxic effects. Therefore, the required tests remain the appropriate follow-up for the reliable positive *in vitro* MNT and chromosome aberration assays and a change of the test systems is not warranted.

You proposed in your comments to the draft decision a testing strategy. Your testing strategy intends to generate supporting information in order to rank antimony-containing substances for further testing. However, ECHA notes that it does not address the concern that ATO may have genotoxic carcinogenic properties directly, but delays the generation of necessary information. As the concern is already manifest for this Substance, ECHA needs to clarify it as soon as possible. The testing strategy you provided is thus not a suitable alternative to the information request.

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The new information in your dossier from ToxTracker and the bioelution tests provide an uncertain prediction of bioavailability of the test materials. It does not allow for conclusions on properties such as toxicity, uptake, distribution, excretion or metabolism. In conclusion, the additional data provided in the dossier update do not diminish the need for the animal studies requested in this decision for ATO.

Proposals for amendment (PfAs) and consideration of your comments on the PfAs

Two PAs were submitted by one MSCA. The first PfA requested to better explain in the decision that it is your responsibility to ensure that the combination of studies does not impair the validity and the results of the information of each individual study. The second PfA requested to specify the exposure route.

In your comments on the proposals for amendment you agreed to both PfAs.

With respect to the 1st PfA, you commented that: i) additional 50-100 animals will be needed before commencing the full test to perform validation steps; ii) it is not feasible to conduct the study in the time frame given in the draft decision (21 months) and that an additional 6-12 months are required; and iii) there are technical difficulties (such as potential differences in the intensity and duration of ATO exposures and sample preparation for the both studies) that may mean that combined studies are not feasible and that a significant reduction in the number of animals used may not be realized. To justify the extension of the deadline, you describe the challenges and provide an estimated timeframe consisting of four steps that would require three months each:

1) Identify proficient CRO able to support study at competitive price (and with early availability;
2) Validation work for analytical work and preparation of dose range and limit studies,
3) Preliminary study;
4) Main study design.

Considering that the set-up of the combined study is indeed not trivial, ECHA agrees to extend the deadline by an additional six months. More specifically, it acknowledges that additional time may be needed for the validation work and for developing the design of the main study (steps 2 and 4). No extra time is considered needed for steps 1 and 3, as the original deadline of 21 months already includes the time for the preliminary studies (step 3) and three months' time provided for the registrants to agree who will perform the required test may be also used for the purpose of finding a proficient CRO.

Furthermore, in case you demonstrate that it was not possible to overcome the technical challenges and that the conduct of the combined studies OECD TG 474 and 489 is not feasible or would not result in a reduced number of animals used, you may perform the two studies separately, each with the specifications described in this decision.

Conclusion

Therefore, in accordance with Article 46(1) of the REACH Regulation, you must carry out the following study using the Substance subject to this decision: Combined *in vivo* mammalian alkaline comet assay (test method: OECD TG 489) with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474)

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in mice, inhalation (nose only) route. The comet assay must be performed on the following tissues: nasal mucosa, alveolar epithelium, bronchoalveolar lavage cells, and liver parenchyma with and without additional specific investigation on oxidative DNA damage for each of the tissues mentioned. The erythrocytes used for OECD TG 474 must be derived from peripheral blood. You may perform the two studies separately, with the specifications as above, if the conduct of combined studies is not feasible or would not result in a reduced number of animals used. In such circumstances justification must be provided.

Notes for consideration

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

You may consider examining gonadal cells, as this would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the Substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

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**Appendix 2: Procedural history**

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to carcinogenicity, wide dispersive use, high RCR, exposure of workers, and high aggregated tonnage, diantimony trioxide CAS No 1309-64-4 (EC No 215-175-0) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2018. The updated CoRAP was published on the ECHA website on 20 March 2018. The competent authority of Germany (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding repeated dose toxicity, mutagenicity and reproductive toxicity.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision under Article 46(1) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 20 March 2019.

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

**Registrant(s)' commenting phase**

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took the comments from you which were sent within the commenting period as well as a dossier update received on 20 June 2019 into account. They are reflected in the reasons (Appendix 1). The requests were not amended.

**Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee**

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision and modified the draft decision. They are reflected in the reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

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

Your comments on the proposals for amendment were taken into account by the Member State Committee.

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**MSC agreement seeking stage**

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-68 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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**Appendix 3: Further information, observations and technical guidance**

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

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

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

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who will carry out the study on behalf of the other registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at: https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx?CaseNumber=SEV-215-175-0-1

   Further advice can be found at: http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the registrants to perform the stud(y/ies) on behalf of all of them.