EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 13 January 2021

Addressees
Registrants of JS_sodium_dithionite as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
14/01/2020

Registered substance subject to this decision ("the Substance")
Substance name: Sodium dithionite
EC number: 231-890-0
CAS number: 7775-14-6

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***20 April 2022***.

Requested information must be generated using the Substance unless otherwise specified.

A. Information required from all the Registrants subject to Annex VII of REACH

1. Skin sensitisation (Annex VII, Section 8.3.)

   i. *in vitro*/in chemico skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD TG 442D) and activation of dendritic cells (OECD TG 442E) (Annex VII, Section 8.3.1.) with the Substance; and
   ii. *in vivo* skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429) with the Substance, in case the in vitro/in chemico test methods specified under point i) are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment;

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), with the Substance using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102

B. Information required from 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)

2. If negative results are obtained in tests performed for the information requirement of Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. then: In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490)

Reasons for the requests are explained in the following appendices:

- Appendix entitled "Reasons common to several requests";
- Appendices entitled "Reasons to request information required under Annexes VII to VIII of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[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 Reasons common to several requests

1. Assessment of your read-across approach under Annex XI, Section 1.5.

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

- Skin sensitisation (Annex VII, Section 8.3.)
- In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)
- In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- In vivo mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2)
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

ECHA has considered the scientific and regulatory validity of your read-across approach(es) 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. 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 (addressed under 'Assessment of prediction(s)').

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance[2] and related documents[3, 4].

A. Predictions for toxicological properties

You have provided a read-across justification document in the IUCLID Sections 7.4, 7.6 and in the CSR.

You read-across between the following substances, as source substances:

- Sodium sulphite; disodium sulfite, EC No. 231-821-4 (CAS RN 7757-83-7);
- Ammonium thiosulphate; diammonium thiosulfate, EC No. 231-982-0 (CAS RN 7783-18-8);
- Disodium disulphite, EC No. 231-673-0 (CAS RN 7681-57-4);
- Dipotassium disulphite, EC No. 240-795-3 (CAS RN 16731-55-8);
- Sodium hydrogen sulphite, Ec No. 231-548-0 (CAS RN 7631-90-5);
- Sodium thiosulphate pentahydrate, EC No. 600-156-5 (CAS RN 10102-17-7)

and the Substance as target substance.

---

[2] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals. 2008 (May) ECHA, Helsinki. 134. pp. Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

[3] Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)

[4] Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017 (March) ECHA, Helsinki. 40 pp. Available online: https://doi.org/10.2823/794394

You have provided the following reasoning for the prediction of toxicological properties: *"A comprehensive read-across concept has been developed for sulfites, hydrogensulfites and metabisulfites, based on the pH-dependant equilibrium in aqueous solutions which is summarised in the following equations:*

SO2+ H2O <-> `H2SO3´ H2SO3<-> H++ HSO3-<-> 2H++SO32- 2HSO3-<->H2O +S2O52-

In consequence, under most physiological circumstances, sulfite and hydrogensulfite anions will be present in almost equimolar quantities, irrespective of their origin either as sulfites, hydrogensulfites and metabisulfites. Unrestricted read-across between the groups of sulfites, hydrogensulfites and metabisulfites is therefore considered justified. Since the nature of the cations such as sodium, potassium and ammonium is not assumed to contribute substantially to differences in toxicity and solubility (all compounds are very soluble in water), only the chemical and biological properties of the anionic sulfite moiety are considered as relevant determinants. Further, it is well established that sodium dithionite is unstable in water, thereby disproportionating to form sodium hydrogen sulfite and sodium thiosulfate (equation II)[1], so that this substance is also considered to be covered by the read-across concept described above." [...]

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which is based on the formation of common (bio)transformation products. The properties of your Substance are predicted to be quantitatively equal to those of the source substance. The crucial aspect of your hypothesis is rapid hydrolysis of dithionite under all relevant conditions to form thiosulphate and sulphite which may then convert to other species depending on the pH.

ECHA has identified the following issues with regards to your predictions of toxicological properties:

i. 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"*[5]. 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).

As indicated above, your read-across hypothesis is based on the assumption that common (bio)transformation products are formed. In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substances, and, if relevant, information on the properties of the non-common compound(s) are necessary to confirm that they cause the same type of effects.

The data set in you dossier does not include reliable information on the toxicological properties of the Substance. No toxicokinetic data on the Substance is provided in your registration dossier. The dossier only contains non guideline toxicokinetic data on the analogues sulphur dioxide, sulphite and sodium sulphite (no EC No.s reported).

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

You argue that dithionate will rapidly hydrolyse under relevant conditions to form thiosulphate and sulphite which may then convert to other species depending on the pH. The only information available in your dossier is a hydrolysis study performed at pH 8.5. and 50 degrees.

Consequently, there is no information in your dossier on whether hydrolysis of dithionate proceeds rapidly at all pHs relevant to the information requirements for which you read across (specifically ~pH 7 which is relevant for the information requirements subject to read across). There is information available (ullmann's encyclopedia of industrial chemistry) to suggest that the hydrolysis is not as rapid at neutral pH and consequently there may be significant exposure to dithionite upon administration. You have not addressed this aspect in your read across justification.

Finally, for the reasons explained under the corresponding sections below (ii., 2., and Appendices A. and B.), some of the studies on the source substance are not adequate to fulfil the corresponding information requirement.

Your technical dossier does not include relevant, reliable and adequate information for the Substance and of the source substances to support your read-across hypothesis. In the absence of such information, you have not established that the Substance and of the source substance(s) are likely to have similar properties. Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

ii. *Read-across hypothesis contradicted by existing 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[6] 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 substances. The observation of differences in the toxicological properties between the source substances and the Substance 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.

Your read-across hypothesis is based on the assumption that the structurally similar target and source substances cause the same type of effects. However, this hypothesis is contradicted by existing data submitted for different properties.

- Concerning *In vitro* chromosomal aberration in mammalian cells

  The results of the information on mutagenicity obtained with the source substances vary. Specifically, positive results are observed in the *in vitro* chromosomal aberration study conducted with sodium hydrogen sulphite (EC number 231-548-0) and dipotassium disulphite (EC number 240-795-3) while negative results are reported for equivalent studies conducted with disodium disulphite (EC number 231-673-0) and sodium hydrogen sulfite (EC number 231-548-0).

- Concerning *In vivo* chromosomal aberration in mammalian cells

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

Similarly, positive results are observed in the chromosomal aberration and micronucleus studies performed with the disodium disulphite, EC No number. 231-673-0 (CAS RN 7681-57-4) and dipotassium disulphite, EC number. 240-795-3 (CAS RN 16731-55-8) while negative results have been obtained with sodium sulphite, EC number 231-821-4 (CAS RN 7757-83-7).

- Concerning *In vitro* gene mutation in bacterial and mammalian cells

  The results of the *in vitro* gene mutation test in bacterial cells with disodium disulphite, EC number 231-673-0 (CAS RN 7681-57-4) are both negative and positive while the results with Sodium thiosulphate pentahydrate, EC number 600-156-5 (CAS RN 0102-17-7) of the *in vitro* gene mutation in bacterial cells are negative. Similarly, for the gene mutation in mammalian cells the results for sodium hydrogen sulphite, EC number 231-548-0 (CAS RN 7631-90-5) are negative while the results with sodium hydrogensulfite, EC number 231-548-0 (CAS RN 7631-90-5) and sodium sulfite disodium sulphite, EC number 231-821-4 (CAS RN 7757-83-7) are concluded as ambiguous.

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

B. Conclusions on the read-across approach

As explained above, you have not established that relevant properties of the Substance can be predicted from data on the analogue substance. Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. and your grouping and read-across approach is rejected.

2. Assessment of your weight-of-evidence adaptation under 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 or in vitro micronucleus study (Annex VIII, Section 8.4.2.)
- In vivo mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2)
- In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

Your weight of evidence adaptation raises the same decifiencies irrespective of the information requirement for which it is invoked. Accordingly, ECHA addressed these deficiencies in the present Appendix, before assessing the specific standard information requirements in the following appendices.

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.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight-of-evidence approach.

However, for each relevant information requirement, you have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has or has not a particular dangerous property.

This deficiency affects the weighing of the sources of information 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.

In spite of this critical deficiency, which in itself could lead to the rejection of the adaptation, ECHA has nevertheless assessed the validity of your adaptation.

In that context , we identified the following issue recurrent for all the information requirements relying on a weight of evidence adaptation:

Reliability of the read across approach

Section A of the present Appendix identifies deficiencies of the grouping and read across approach used in your dossier. These findings apply equally to the sources of information relating to analogue substances submitted under your weight of evidence adaptations.

Additional issues related to weight of evidence are addressed under the corresponding information requirements in the following Appendices.

Appendix A: Reasons to request information required under Annex VII of REACH

1. Skin sensitisation(Annex VII, Section 8.3.)

Skin sensitisation is a standard information requirement in Annex VII, Section 8.3. to the REACH Regulation. Column 1 of Section 8.3. requires the registrants to submit information allowing a conclusion whether the substance is a skin sensitiser and whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and risk assessment, where required.

You have provided the following information in the technical dossier, based on which you conclude that the Substance is not a skin sensitiser:

i. *in vivo* Local Lymph Node Assay (key study, OECD TG 429, [redacted], 2010),
ii. three *in vivo* Local Lymph Node Assays (key studies, OECD TG 429, [redacted], 2010) with the analogues, disodium disulphite, EC number 231-673-0 (CAS RN 7681-57-4, sodium sulphite, EC number 231-821-4 (CAS RN 7757-83-7) and diammonium thiosulfate, EC number 231-982-0 (CAS RN 7783-18-8).

You have submitted an Annex VII, Section 8.3., Column 2 adaptation for not performing the *in vitro* studies i.e. *"an in vitro or in chemico skin sensitisation study does not need to be conducted because adequate data from an in vivo skin sensitisation study are available"*.

To fulfil the information requirement, as specified in the Annex VII, Section 8.3., Column 1 to the REACH Regulation, the following aspects must be covered:
1. whether the Substance causes skin sensitisation, and
2. whether the Substance can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), in case, the Substance is considered to be a skin sensitiser.

1. Assessment whether the Substance causes skin sensitisation

You have provided an OECD TG 429 study on the Substance according to the Local Lymph Node Assay test method. You further explain that *"The test was performed in accordance with the method according to Ehling et al (2005): An european inter-laboratory validation of alternative endpoints of the murine local lymph node assay: first round, Toxicology 212 (2005) 60-68 and Ehling et al (2005): An european inter-laboratory validation of alternative endpoints of the murine local lymph node assay: 2nd round, Toxicology 212 (2005) 69-79"*.

We have assessed this information and identified the following issue(s):

To fulfil the requirements of OECD TG 429:
- The highest concentration must be the highest technically possible concentration that maximises exposure while avoiding systemic toxicity and/or excessive local skin irritation (OECD TG 429, paragraph 18)
- Wholly aqueous vehicles are to be avoided (OECD TG 429, paragraph 19).

However, in the studies you submitted:
- No dose level selection rationale was provided for selecting the highest dose (e.g. 50% in vehicle aqua *ad iniectabilia*)
- A wholly aqueous vehicle was used without incorporation of an appropriate solubilizer (e.g. 1% Pluronic® L92).

In your comments on the draft decision you stated that you consider the *in vivo* study (i.) available in the registration dossier to be valid. However, you do not substantiate your comment with any scientific information and you do not address any of the deficiencies highlighted above. Nevertheless, you agreed to provide a new *in vivo* study on the endpoint skin sensitisation.

For the three analogues (studies ii.) the same test design is used and therefore the study has the same deficiencies as identified above ([redacted], 2010). In addition, for the reasons explained under section 1. of the 'Appendix on Reasons common to several requests', your read-across adaptation is rejected.

Therefore the studies do not fulfil the key parameters set in the OECD TG 429 and do not allow to conclude on whether the Substance causes skin sensitisation.

2. Assessment whether the Substance can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

As the currently available data does not allow to conclude whether the Substance causes skin sensitisation, this condition cannot be assessed.

Considering the conclusions of the assessments under points 1. and 2. above your adaptation is rejected and the information requirement is not fulfilled.

Information on the study design

To fulfil the information requirement for the Substance for skin sensitisation, *in vitro/in chemico* studies (OECD TG 442C, 442D and 442E) are considered suitable. In case *in vitro/in chemico* methods are not suitable for the Substance or the results cannot be used for classification and risk assessment an *in vivo* skin sensitisation study must be performed and the murine local lymph node assay (LLNA) (OEDC TG 429) is considered as the appropriate study.

In your comments on the draft decision, you argued that the available *in vitro* test systems for skin sensitisation are not relevant for inorganic substances forming anionic species in aquatic media. You also claim that all assays OECD 442 (C, D and E) have their limitations as *in vitro* methods for sodium dithionite, primarily because this substance is a reducing agent which will not modify cysteine residues (OECD 442 C and D), thereby yielding by default negative responses. While ECHA considers your rationale for not performing the in vitro studies due to the properties of the Substance plausible, this information must be submitted in the registration dossier.

Moreover, you have proposed to omit the *in vitro* testing stage and directly repeat the *in vivo* OECD 429 test (LLNA, modified according to Ehling[1]) and include the use of an additional solubiliser to reduce the surface tension caused by ionic species to maximise the contact area on the skin. Concerning this modification proposed by you (OECD TG 429 according to Ehling), ECHA notes, that you have not provided any justification as to why modifications to the OECD TG 429 are considered necessary based on the properties of the Substance. ECHA notes that the current OECD TG 429 has incorporated measurements of ear thickness that can be used to distinquish between irritant and skin sensitising reactions. Therefore, based on the information available in your dossier and your comments, the requested study must normally be performed according to the internationally adopted method(s) i.e. OECD TG 429.

Regarding the use of an additional solubiliser you also stated that the OECD 429-validated solubiliser Pluronic® L92 is not available in Europe and that an alternative solubiliser needs

to be selected. You also called for a decision by ECHA on which solubiliser should be used for the experimental setup in order to ensure regulatory acceptance of the test repeat.

ECHA notes that Pluronic® L92 is given as an example in the OECD TG 429 (paragraph 19). It is your responsibility to identify the most suitable solubiliser for you Substance in order to achieve the objective pursued by the specifications identified in the OECD TG 429.

2. *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 this standard information requirements by applying weight-of-evidence approaches in accordance with Annex XI, Section 1.2.

In support of your adaptation you have provided the following source of information with the Substance:

i. An *in vitro* gene mutation study in bacteria (reliability 2, OECD TG 471, [redacted], 1989) with the following four strains, TA 98, TA 100, TA 1535, and TA 1537.

In addition, you have provided several sources of information from studies performed with analogues:

ii. Three *in vitro* gene mutation studies in bacteria (reliability 2, OECD TG 471, [redacted], 1989) performed with the analogues sodium sulphite (EC number 231-821-4, CAS RN 7757-83-7), dipotassium sulfite (EC number 233-321-1, CAS RN 10117-38-1), sodium thiosulphate pentahydrate (EC number,600-156-5, CAS RN 10102-17-7) and ammonium thiosulfate (EC number 231-982-0, CAS RN 7783-18-8) with four strains;
iii. An *in vitro* gene mutation study in bacteria (reliability 2, similar to OECD TG 471, [redacted], 1978) performed with the analogue disodium disulphite (EC number 231-673-0, CAS RN 7681-57-4), with five recommended strains;
iv. An in vitro gene mutation study in bacteria (reliability 1, OECD TG 471, [redacted], 2001) with the analogue Ammonium thiosulfate (EC number 231-982-0, CAS RN 7783-18-8) with five recommended strains;
v. An *in vitro* gene mutation studies in bacteria (reliability 2, similar to OECD TG 471, [redacted], 1979) performed with the analogue Sodium thiosulphate pentahydrate (EC number 600-156-5, CAS RN 10102-17-7) with five recommended strains;
vi. Two non-guideline *in vitro* gene mutation studies in bacteria (reliability 2, similar to OECD TG 471, Ishidate, 1984) performed with the analogues disodium disulphite (EC number 231-821-4, CAS RN 7757-83-7), and dipotassium disulphite (EC number 240-795-3, CAS RN 16731-55-8) in strains TA 1535, TA 1537, TA 98, TA 100, TA 92 and TA 94 (not the correct 5 strains);
vii. A non guideline *in vitro* gene mutation study in bacteria (reliability 3, Münzer, 1980, publication) with the analogue sodium hydrogen sulfite (EC number 231-548-0, CAS RN 7631-90-5) in four strains.
viii. A non guideline *in vitro* gene mutation study in bacteria (reliability 3, Pagano, 1990) with the analogue disodium disulphite (EC number 231-673-0, CAS RN 7681-57-4) in one strain;
ix. A non guideline *in vitro* gene mutation study in bacteria (reliability 3, Pagano & Zeiger, 1987, publication) with the analogue disodium disulphite (EC number 231-673-0, CAS RN 7681-57-4) not with the correct five strains;
x. A supporting *in vitro* gene mutation study in bacteria (reliability 3, similar to OECD TG 471, [redacted], 1975) with the analogue (EC 231-821-4, CAS RN 7757-83-7).

To fulfil the information requirement, normally a study performed according to OECD TG 471 must be provided. OECD TG 471 requires the study to investigate gene mutations in bacteria using 5 different bacterial strains.

The sources of information (i.) to (x.) may provide relevant information on *in vitro* gene mutations in bacteria.

However, the reliability of these sources of information is significantly affected by the following deficiencies:

1) One of the specifications of OECD TG 471 (1997) includes that 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). The reported data for source of information (i.) with the Substance you have provided did not include results for the required fifth strain, S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). Therefore this source of information does not investigate the specificity of the fifth strain with regards to the types of mutation that can be induced. As indicated in OECD TG 471, this information is required as the fifth strain may detect certain oxidising mutagens, cross-linking agents and hydrazines, which the other four strains cannot detect. Therefore, in absence of information of the fifth strain the provided study cannot be considered as a reliable source of information that could contribute to the conclusion on on gene (point) mutations in the five bacterial strains.

2) Information from source substance(s) can contribute to a weight of evidence adaptation only if the read-across is acceptable.
Studies (ii.) to (x.) are performed with analogue substances.
However, for the reasons explained under Appendix on Reasons common to several requests, there are deficiencies identified with the read across adaptation. These deficiencies affect significantly the reliability of the sources of information relating to analogue substances and relied upon in your weight of evidence adaptation. Therefore, the sources of information (ii.) to (x) cannot contribute to the weight of evidence adaptation.

In addition, the reliability of the sources of information (ii.) and (vi.) to (x.) is also affected by the following issue:

3) Although you do not explicitly claim an adaptation, ECHA understands that sources of information (ii.) and (vi.) to (x.) were submitted in order to meet the information requirement by means of adaptation according to Annex XI, Section 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods.

   The adaptation rule in Annex XI, Section 1.1.2. imposes a number of cumulative conditions for an adaptation to be valid, in particular:
   - Adequate and reliable documentation of the study is provided.

   However, you have not provided adequate and reliable documentation in a form of a robust study summary, as required by Article 10(a)(vii) and Article 3(28). You have also assigned a reliability score of 3 (not reliable) to these sources of information (vii.) to (x.) due to limited information provided on the study. Moreover, these non guideline and non GLP studies do not include the recommended five strains.

Taken together, even if these sources of information provide information on gene mutations in bacteria, their reliability is affected so significantly by the deficiencies as described above in points 1) to 3) that they cannot be taken into consideration in a weight of evidence approach.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 471. Therefore, your adaptation is rejected and the information requirement is not fulfilled. In your comments on the draft decision, you agree to perform the requested study.

Information on the study design

To fulfil the information requirement for the Substance, the *in vitro* gene mutation study in bacteria (OECD TG 471) should be performed using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

Appendix B: Reasons to request information required under Annex VIII of REACH

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

An *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is a standard information requirement in Annex VIII to REACH.

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

In support of your adaptation you have provided the following sources of information with analogue substances:

In vitro studies

i. a GLP study similar to OECD TG 473 with ammonium thiosulphate (EC No. 231-982-0, CAS RN 7783-18-8) (reliability 1, [redacted], 2001);

ii. nine other *in vitro* chromosomal aberration and micronucleus studies (non guideline, non GLP, reliability 3) with hydrogen sulphite (EC No. 231-548-0, CAS RN 7631-90-5), disodium disulphite (EC No. 231-673-0, CAS RN 7681-57-4) and dipotassium disulphite (EC No. 240-795-3, CAS RN 16731-55-8).

In vivo studies

iii. an OECD TG 474 GLP study using Sodium sulphite (EC No. 231-821-4, CAS RN 7757-83-7) (reliability 1, [redacted], 2008) via subcutaneous route and two other *in vivo* micronucleus studies with disodium disulphite (EC No. 231-673-0, CAS RN 7681-57-4) (non guideline, non GLP, reliability 3) ;

iv. three *in vivo* chromosomal aberration studies (non guideline, non GLP, reliability 3), with analogue substances;

v. a comet assay (non guideline, non GLP, reliability 3) performed with an analogue substance.

To fulfil the information requirement, normally a study performed according to OECD TG 473/487 must be provided. OECD TG 473/487 investigate the following:

- Detection and quantification of structural or numerical chromosomal aberrations in cultured mammalian cells including data on the cytotoxicity and the frequency of cells with chromosomal aberrations or micronuclei.

The sources of information (i.) to (v.) may provide relevant information on structural or numerical chromosomal aberrations in cultured mammalian cells.

However, the reliability of these sources of information is significantly affected by the following deficiencies:

1) Information from source substance(s) can contribute to weight of evidence adaptation only if the read-across is acceptable.
Studies (i) to (v.) are performed with analogue substances.
However, for the reasons explained under Appendix on Reasons common to several requests, there are deficiencies identified with the read-across adaptation. These deficiencies affect significantly the reliability of the sources of information relating to analogue substances and relied upon in your weight of evidence adaptation. Therefore the sources of information (i.) to (v.) cannot contribute to the weight of evidence adaptation.

In addition, the reliability of the sources of information is also affected by the following issues:

2) The specifications of OECD TG 473/487, include the following:

   a) At least 300 well-spread metaphases (OECD TG 473)
   b) Data on the cytotoxicity and the frequency of cells with structural chromosomal aberrations / micronuclei for the treated and control cultures must be reported.

However, the reported data for study (i.) you have provided does not include:

a) the scoring of at least 300 metaphases per concentration (OECD TG 473). You reported that 200 metaphase spreads (100 per duplicate flask) were scored.
b) data on the cytotoxicity and/or the frequency of cells with structural chromosomal aberration(s) for the treated and control cultures (OECD TG 473).

As indicated in OECD TG 473 this information is required to conclude whether a test chemical is clearly negative. Therefore the acceptability criteria of the OECD TG 473 are not met and the provided study cannot be considered as a reliable source of information that could contribute to the conclusion on this information investigated by the required study.

3) Although you do not explicitly claim an adaptation, ECHA understands that sources of information iii. to v. were submitted in order to meet the information requirement by means of adaptation according to Annex XI, Section 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods.

   The adaptation rule in Annex XI, Section 1.1.2. imposes a number of cumulative conditions for an adaptation to be valid, in particular, adequate and reliable documentation of the study must be provided.

   However, for sources of information provided under (iii.) to (v.) you have not provided adequate and reliable documentation in a form of a robust study summary, as required by Article 10(a)(vii) and Article 3(28). You have also assigned a reliability score of 3 (not reliable) to these non guideline, non GLP, sources of information due to limited information provided on the study.

4) To investigate intrinsic properties of the Substance for detection and quantification of structural or numerical chromosomal aberrations, the selected route of exposure must aim to maximise the internal exposure to the Substance.

   The source of information (iii.) is a study using subcutaneous injection. This route of administration is not reliable route of administration to investigate the structural or numerical chromosomal aberrations because the toxicokinetics regarding absorption, distribution, metabolism and elimination is not similar to that with oral route of administration and the internal exposure is very different. Therefore, source of information (iii.) is not reliable and does not contribute to the weight of evidence adaptation.

In the absence of reliable information on the elements investigated by the study normally required no conclusion can be drawn on structural or numerical chromosomal aberrations in cultured mammalian cells as required by the information requirement.

Taken together, even if these sources of information provide information on structural or numerical chromosomal aberrations in cultured mammalian cells, their reliability is affected so significantly by the deficiencies as described above in in points 1) to 4) that they cannot be taken into consideration in a weight of evidence approach.

On the basis of the information provided in the Appendix on Reasons common to several requests and on the above, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an OECD TG 473 or 487 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled. In your comments on the draft decision, you agree to perform the requested study.

Study design

To fulfil the information requirement for the Substance, either *in vitro* cytogenicity study in mammalian cells (OECD TG 473) or *in vitro* micronucleus study (OECD TG 487) are considered suitable.

2. Only if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. is obtained, In vitro gene mutation study in mammalian cells

An *in vitro* gene mutation study in mammalian cells is an 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.

Triggering of the study

Your dossier contains an adaptation (weight-of-evidence) for an *in vitro* gene mutation study in bacteria, and an adaptation (weight-of-evidence) 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.2 and B.1 of this draft decision.

The result of the requests for information in A.2 and B.1 of this decision 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.

Information in dossier

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

In support of your adaptation you have provided the following sources of information with analogue substances:

i. an OECD TG 476 study with disodium disulphite (EC No. 231-673-0, CAS RN 7681-57-4)(reliability1, [redacted], 2010);
ii. an OECD TG 476 study with diammonium thiosulfate (EC No. 231-982-0, CAS RN 7783-18-8)(reliability 1, [redacted], 2010);
iii. two other reliability 3 studies (non guideline non GLP) with sodium hydrogen sulphite (EC No. 231-548-0, CAS RN 7631-90-5).

To fulfil the information requirement, normally a study performed according to OECD TG 476/490 must be provided. OECD TG 476/490 investigate the following:

- Detection and quantification of gene mutations (point mutations, frame-shift mutations, small deletions, etc.) in cultured mammalian cells including data on the frequency of mutant colonies.

The sources of information (i.) to (iii.) may provide relevant information on *in vitro* gene mutations in cultured mammalian cells.

However, the reliability of these sources of information is significantly affected by the following deficiencies:

1) Information from source substance(s) can contribute to weight of evidence adaptation only if the read-across is acceptable.

   Studies (i), (ii.) and (iii.) are performed with analogue substances. However, for the reasons explained under Appendix on Reasons common to several requests, there are deficiencies identified with the read-across adaptation. These deficiencies affect significantly the reliability of the sources of information relating to analogue substances and relied upon in your weight of evidence adaptation.Therefore the sources of information (i.) to (iii.) cannot contribute to the weight of evidence adaptation.

2) Moreover, although you do not explicitly claim an adaptation, ECHA understands that sources of information provided under iii. were submitted in order to meet the information requirement by means of adaptation according to Annex XI, Section 1.1.2. This adaptation rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods.

   The adaptation rule in Annex XI, Section 1.1.2. imposes a number of cumulative conditions for an adaptation to be valid, in particular adequate and reliable documentation of the study must be provided.

   However, you have not provided adequate and reliable documentation in a form of a robust study summary, as required by Article 10(a)(vii) and Article 3(28). You have also assigned a reliability score of 3 (not reliable) to these non guideline, non GLP, sources of information due to limited information provided on the study.

Taken together, even if these sources of information (i. to iii.) provide information on gene mutations in mammalian cells, their reliability is affected so significantly for the reasons explained in 1) and 2) that they cannot be taken into consideration in a weight of evidence approach.

On the basis of the information provided in the Appendix on Reasons common to several requests and on the above, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an OECD TG 476 or 490 study. Therefore, your adaptation is rejected and the information requirements is not fulfilled. In your comments on the draft decision, you agree to perform the requested study.

Study design

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

Appendix C: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

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

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

3. 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 on How to report robust study summaries[7].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity 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.

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[8].

[7] https://echa.europa.eu/practical-guides
[8] https://echa.europa.eu/manuals

Appendix D: Procedure

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

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 30 October 2019.

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

In your comments you agreed to the draft decision. ECHA took your comments into account and 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 E: List of references - ECHA Guidance[9] and other supporting documents

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 where relevant.

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 where relevant.

Read-across assessment framework (RAAF, March 2017)[10]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[11]

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.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents[12]

[9] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[10] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[11] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316
[12] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix F: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.