

Helsinki, 25 April 2022

Addressees

Registrant(s) of Reactive Orange 72/78 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision

08/06/2020

Registered substance subject to this decision ("the Substance")

Substance name: 7-acetamido-4-hydroxy-3-[[4-[[2-(sulphooxy)ethyl]sulphonyl]phenyl]azo]naphthalene-2-sulphonic acid, sodium salt

EC number: 287-574-8

CAS number: 85536-87-4

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 **31 July 2024**.

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.) with the Substance
 - 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 (EU B.71/OECD TG 442E) (Annex VII, Section 8.3.1.); and
 - ii. Only if 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, *in vivo* skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429);
2. *In vivo* genotoxicity study, as requested below, in B.2.

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. *In vivo* genotoxicity study to be selected according to the following scenarios:
 - a. If the test results of request B.1 are negative:

In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum, with the Substance

OR

Transgenic rodent somatic and germ cell gene mutation assays (Annex IX, Section 8.4., column 2; test method EU B.58./OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver, glandular stomach, with the Substance; duodenum must be harvested and stored for up to 5 years. The duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

b. If the test results of request B.1 are positive:

In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route, with the Substance. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

3. Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.) to be combined with the Screening for reproductive/developmental toxicity below
4. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) by oral route, in rats
5. Hydrolysis as a function of pH (Annex VIII, Section 9.2.2.1.; test method: EU C.7./OECD TG 111) – test under slightly alkaline conditions (i.e., covering only pH values between 7 and 8.5 and at least pH values of 8 and 8.5).

Reasons for the request(s) 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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa.

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

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

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". 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.

Authorised¹ under the authority of Mike Rasenberg, 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

i. Assessment of the Grouping of substances and read-across approach under Annex XI, Section 1.5.

You seek to adapt the following standard information requirements by applying (a) read-across approach(es) in accordance with Annex XI, Section 1.5:

- Skin sensitisation (Annex VII, Section 8.3.)
- 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)
- Short-term repeated dose toxicity (28 days) (Annex VIII, Section 8.6.1)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1)

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.

Grouping of substances and read-across approach

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 below).

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance R.6. and related documents^{2,3}.

A. Predictions for toxicological properties

You have provided a read-across justification document in IUCLID Section 13.

You read-across between the structurally similar substances, Reactive orange 16, EC No. 243-653-9, Reaktiv-Orange FD 19969 FW, EC No. 404-600-7 and Reactive Blau FC 05717, EC No. 401-560-2 as source substances and the Substance as target substance.

Your reasoning for the prediction of toxicological properties is based on "*similar cleavage products*". Reference is made to "*azo reductase breakdown*." The toxicokinetic studies, which you have provided, concern Reactive Black, which is not the source substance for the read-across of the toxicological endpoints addressed in this decision.

Your read-across justification document gives only generic information on hydrolysis and cleavage of azo substances. No experimental data on the rate of transformation and cleavage of the Substance or that of the source substances has been provided.

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The

² Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: [Read-Across Assessment Framework \(https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across\)](https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)

³ 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>

properties of your Substance are predicted to be quantitatively equal to those of the source substance.

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

1. 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*"⁴. 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). Supporting information must include bridging studies to compare properties of the Substance and source substances.

Missing information on the formation of common and non-common compounds

As indicated above, your read-across hypothesis is based on the transformation of the Substance and of the source substance(s) to a common compound(s). In this context, information characterising the rate and extent of the transformation of the Substance and of the source substances is necessary to confirm the formation of the proposed common transformation products and to assess the impact of the exposure to the parent compounds as well as the impact of non-common dissociation products.

In your dossier, you claim that the Substance and the source substances of the read-across have "*similar cleavage products*". However, you have provided toxicokinetic studies on a substance, Reactive Black, which is neither one of the source substances nor the Substance and you have not provided experimental data to demonstrate similarity of the transformation products and rates of these cleavage of these substances. You also refer to "*similar cleavage products*" without adequate further clarification in your dossier.

In the comments to the initial draft decision, you argue that the information requirements for several requests of this decision are fulfilled by the available studies with source substances in the dossier. In support of your read-across adaptation you provide an updated justification document with additional experimental information on the toxicokinetic behaviour of another representative source substance, which is a close analogue with high structural similarity. In addition, you provide a comparison of physicochemical and (bio)degradation properties of the Substance and the source substances, which were obtained by modelling (*in silico*).

The results of the toxicokinetic studies are in agreement with the modelled information which is available for the source substances and the Substance. Therefore, the information provided as part of your comments addresses the above issue regarding the supporting information. However, as the information is currently not available in your registration dossier, the data gaps remain. You should submit a robust study summary for the toxicokinetic study with the source study reactive black and the updated justification document in an updated registration dossier by the deadline set in the decision.

2. Adequacy and reliability of source study

⁴ Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results to be read across should:

- be adequate for the purpose of classification and labelling and/or risk assessment;
- have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3).

Related deficiencies are addressed under the individual information requirement specific reasons in Appendices A and B below.

B. Conclusions on the read-across approach

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

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

1. Skin sensitisation

Skin sensitisation is an information requirement under Annex VII to REACH (Section 8.3.). Under Section 8.3., Column 1, the registrants must submit information allowing (1) A) a conclusion whether the substance is a skin sensitizer and B) whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A), and (2) risk assessment, where required.

We understand that you have provided a read-across adaptation using a key study in your dossier:

- in vivo Guinea Pig Maximization test, EU Method B.6/OECD TG 406, with the analogue substance Reactive orange 16, EC No. 243-653-9, performed in 1988.

We have assessed this information and identified the following deficiencies:

Grouping and read-across rejected

Your read-across adaptation in the dossier is not considered acceptable, as explained above in the Appendix on reasons common to several requests.

Your comments on the initial draft decision are addressed in the *Appendix on Reasons common to several requests*. In conclusion, the information provided as part of your comments address this deficiency. However, as the information is currently not available in your registration dossier, the data gap remains. You should submit the information by the deadline set in the decision.

With a view to your comments on the "no assessment of potency", it is pointed out that in the absence of an acceptable documentation of the read-across approach also no acceptable data to meet the information requirement are given. This is why ECHA mentions that to be considered compliant and enable a conclusion in cases where the substance is considered to cause skin sensitisation, the information provided must also allow a conclusion whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

Study design

To fulfil the information requirement for the Substance for skin sensitisation, *in vitro/in chemico* studies (OECD TG 442C, OECD TG 442D and EU Method B.71/OECD TG 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) (EU Method B.42/OECD TG 429) is considered as the appropriate study.

2. In vivo genotoxicity study

Under Annex VII, Section 8.4, column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

The ECHA guidance R.7a⁵ states that following a positive result in an *in vitro* test, "*adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold*

⁵ ECHA Guidance R.7a, section R.7.7.6.3, p.570.

mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary."

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, with the Prival modification (OECD TG 471; [REDACTED], 1989), conducted with the Substance, which raises the concern for gene mutation.

In your comments to the initial draft decision, you argue that the increase in mutant frequency observed in the *S. typhimurium* TA 98 strain in the above study was incidental because it was a marginal effect at 2500 µg/plate with a rather high variability between the three replicates, it was not dose-dependent and not seen at the highest concentration. You further indicate your intention to update your dossier with results from a new Ames test using Prival modifications, with the analogue substance Reactive Orange 16.

However, according to OECD TG 471, "*there are several criteria for determining a positive result, such as a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation system*". You indicate in your dossier that the test using the Prival modification was repeated and that the same increase in the number of revertant colonies was obtained, although you did not provide any further details on the results of this second experiment. Therefore, ECHA considers this reproducible positive result in *S. typhimurium* TA 98 as acceptable.

Furthermore, from the table of results for the first Prival experiment repeat you provided with your comments, ECHA considers that the variability observed at 2500 µg/plate (64 ± 18 mutant colonies per plate) is comparable to that of the negative control (30 ± 11 mutant colonies per plate). ECHA also notes that a concentration-related increase in mutant frequency can be observed in *S. typhimurium* TA 98 up to 2500 µg/plate: the mutant frequency ratio compared to controls was 1.5, 2.1 and 1.7 at 500, 2500 and 5000 µg/plate, respectively. The absence of significant increase at the highest concentration tested could be a sign of cytotoxicity. However, you did not provide details on cytotoxicity results and, due to concentration spacing, no information on mutant frequency between 500 and 2500 µg/plate and between 2500 and 5000 µg/plate is available to properly assess the concentration-effect relationship and possible cytotoxicity at higher doses. Therefore, ECHA considers that the absence of significant increase in mutant frequency at the highest concentration does not remove the concern for gene mutation raised by the results.

Finally, ECHA acknowledges your intention to provide further supporting data with the analogue substance Reactive Orange 16, which are yet to be generated. However, as the information is currently not available in your registration dossier and cannot be assessed, the concern for gene mutation raised by the positive *in vitro* gene mutation study in bacteria with the Substance remains. Therefore, you remain responsible for complying with this decision by the set deadline.

No data from an appropriate *in vivo* somatic cell genotoxicity study is available in the dossier. Moreover, the *in vivo* study submitted in your dossier does not address the gene mutation concern as explained under Section B.2.

ECHA considers that an appropriate *in vivo* follow-up mutagenicity study is necessary to address the concern identified *in vitro*.

For the assessment of the information provided and the specifications of the study to be performed, see the request B.2.

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.

No *in vitro* cytogenicity study has been provided. You have provided a read-across adaptation under Annex XI, Section 1.5 as the basis for adapting this information requirement under Column 2 of Section 8.4.2, using an *in vivo* key study in your dossier:

- Study according to OECD Guideline 474 with an analogue substance "Structural Analogue 01 - Litium salt", which is Reactive orange 16, EC 243-653-9, (Mammalian Erythrocyte Micronucleus Test), performed in 1985.

We have assessed this information and identified the following issue:

Grouping and read-across rejected

Your read-across adaptation in your dossier is not considered acceptable, as explained above in the Appendix on reasons common to several requests.

Based on the above, the information you provided does not fulfil the information requirement.

Your comments on the initial draft decision are addressed in the *Appendix on Reasons common to several requests*. In conclusion, the information provided as part of your comments addresses the incompliance relating to this endpoint. However, as the information is currently not available in your registration dossier, the data gap remains. You should submit this supporting information by the deadline set in the decision.

To fulfil the information requirement for the Substance, both *In vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) and *in vitro* micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487) are considered suitable.

2. *In vivo* genotoxicity study

Under Annex VIII, Section 8.4, column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, with the Prival modification (OECD TG 471; ██████████, 1989), which raise the concern for gene mutation. As explained in Appendix A, Section 2, your comments to the draft decision do not remove this concern.

Also, your dossier contains the following *in vivo* study performed with an analogue

- i. Study according to OECD Guideline 474 with an analogue substance "Structural Analogue 01 - Litium salt", which is Reactive orange 16, EC 243-653-9. (Mammalian Erythrocyte Micronucleus Test) Micronucleus Test in male and female NMRI mice after oral administration, performed in 1985.

We have assessed this information and identified the following issues:

A. Grouping and read-across rejected

Concerning this study, your read-across adaptation is not accepted as explained in the Appendix on Reasons common to several requests.

Your comments on the initial draft decision are addressed in issue i.A.1. of the *Appendix on Reasons common to several requests*. In conclusion, the information provided as part of your comments address this deficiency. However, the information is currently not available in your registration dossier.

In addition, we have identified the following endpoint-specific issue.

B. Provided in vivo study does not clarify the concern

As explained under the Appendix on reasons common to several requests, read-across results must be adequate for classification and labelling and/or risk assessment. According to ECHA Guidance R.7a, the *in vivo* somatic cell genotoxicity study must address the specific concern raised by the *in vitro* positive result. However, the available *in vivo* study you submitted do not address the gene mutation concern raised by the positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471; ██████████, 1989). Therefore, the provided *in vivo* test is not appropriate.

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern(s) identified *in vitro*.

Test selection

According to the ECHA Guidance Chapter R.7a⁶, the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation.

Therefore, the TGR and the comet assay are suitable tests to follow up the concern on gene mutation for the Substance.

This decision, however, also requests an *in vitro* test under Annex VIII Section 8.4.2 (see section B.1), which would raise a concern for chromosomal aberration in case of positive results.

In case there are positive results from the *in vitro* cytogenicity study, requested in section B.1 above, the positive *in vitro* results would indicate a concern for **both** chromosomal aberration and gene mutation.

The *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) can be combined in a single study (see OECD TG 474 para. 37c; OECD TG 489 para. 33; ECHA Guidance R.7a, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations. A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

The combined study, together with the results of the *in vitro* mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing *in vivo* mutagenicity and lack thereof. Furthermore, the combined study can help reduce the number of tests

⁶ ECHA Guidance Chapter R.7a, Section R.7.7.6.3

performed and the number of animals used while addressing (structural and numerical) chromosomal aberration as well as gene mutation.

Therefore, in the event of a positive result in the requested *in vitro* cytogenicity study, the comet assay combined with the MN test is the most appropriate study for the Substance.

Test design

In case the comet assay is appropriate and you decide to perform this test, according to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

In line with the OECD TG 489, the test must be performed by analysing tissues from liver, as primary site of xenobiotic metabolism, glandular stomach and duodenum, as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

In case the *in vivo* mammalian alkaline comet assay combined with *in vivo* mammalian erythrocyte micronucleus test (combined OECD TG 489 and OECD TG 474) is appropriate, the test must be performed in line with the test method OECD TG 489. As explained above, the test must be performed in rats, by the oral route, and by analysing comets in tissues from the liver, glandular stomach and duodenum. The combination of OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al.* 2011⁷).

In case the TGR assay is appropriate and you decide to conduct this test, according to the test method EU B.58/OECD TG 488, the test must be performed in transgenic mice or rats and the Substance is usually administered orally.

Based on the recent update of OECD TG 488 (2020), you are requested to follow the new 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.

According to the test method EU B.58/OECD TG 488, the test must be performed by analysing tissues from liver, as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum, as rapidly proliferating tissue and site of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below -70°C) until the analysis of the liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and the liver are negative or inconclusive.

⁷ Bowen D.E. et al. 2011. Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Mutation Research* 722 7–19

Germ cells:

In case you decide to perform the comet assay (or comet assay combined with the *in vivo* micronucleus test), you may consider to collect the male gonadal cells collected from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells.

In case you decide to perform the TGR, you may consider to collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488 the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below $-70\text{ }^{\circ}\text{C}$). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ cells.

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.

3. Short-term repeated dose toxicity study (28-day)

A Short-term repeated dose toxicity study (28 days) is a standard information requirement under Annex VIII to REACH. This information may take the form of a study record or a valid adaptation in accordance with either a specific adaptation rule under Column 2 of Annex VIII or a general adaptation rule under Annex XI.

We understand that you have provided a read-across adaptation using the following studies:

1. A sub-acute toxicity study with the test duration of 14 days, with the Substance, performed in 1975,
2. A study according to OECD Guideline 407, with the analogue substance Reactive orange 16, EC No. 243-653-9, performed in 1985,
3. A study according to OECD Guideline 407, with the analogue substance Reaktiv-Orange FD 19969 FW, EC No. 404-600-7, performed in 1998.

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

Grouping and read-across rejected

The adaptation in your dossier is not considered acceptable, as explained above in the *Appendix on Reasons common to several requests*.

Therefore, the information you provided do not fulfil the information requirement.

Your comments on the initial draft decision are also addressed in the above *Appendix on Reasons common to several requests*. In conclusion, the information provided as part of your comments addresses the incompliance relating to this endpoint. However, as the information is currently not available in your registration dossier, the data gap remains. You should submit the information by the deadline set in the decision.

Information on study design

Referring to the criteria provided in Annex VIII, Section 8.6.1, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the substance is a solid, not present in particulate form.

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407), nor for the screening study for reproductive/ developmental toxicity (OECD TG 421 or TG 422), the conduct of a combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) is preferred to ensure that unnecessary animal testing is avoided. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the same time fulfil the information requirement of REACH Annex VIII, 8.6.1 and that of REACH Annex VIII, 8.7.1.⁸

Therefore the study must be performed according to the OECD TG 422, in rats and with oral administration of the Substance.

4. Screening study for reproductive/developmental toxicity

A Screening for reproductive/developmental toxicity study (test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) is a standard information requirement under Annex VIII to REACH, if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant. There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

ECHA understands that you have provided a read-across adaptation using a key study in your dossier:

- A study according to OECD Guideline 415, One-Generation Reproduction Toxicity Study, in rats, with an analogue substance Reactive Blau FC 05717, EC No. 401-560-2, performed in 2002.

We have assessed this information and identified the following deficiencies:

Grouping and read-across rejected

The adaptation in your dossier is not considered acceptable, as explained above in the Appendix on reasons common to several requests.

Therefore, the information you provided do not fulfil the information requirement.

Also your comments on the initial draft decision are addressed in the above *Appendix on Reasons common to several requests*. In conclusion, the information provided as part of your comments addresses the incompliance relating to this endpoint. However, as the information is currently not available in your registration dossier, the data gap remains. You should submit the information by the deadline set in the decision.

Information on study design

⁸ ECHA Guidance, Section R.7.6.2.3.2., pages 484 to 485 of version 6.0 – July 2017.

(https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf)⁹ ECHA Guidance R.7a, Section R.7.6.2.3.2.¹⁰ The pH of domestic wastewater is typically between 6–8 but is largely related to the alkalinity of the carriage water. In areas having soft water (alkalinity between 50 and 100 mg/L as CaCO₃), the pH of domestic wastewater is around 6.0 to 6.5. In areas having moderately hard water (alkalinity between 100 and 300 mg/L as CaCO₃) it is between 7.0 and 8.0. In areas having hard water (alkalinity higher than 300 mg/L as CaCO₃) it is between 7.5 and 9.0. Some industrial wastewaters can be quite acidic or alkaline. The optimum pH range for aerobic biodegradation lies between 6.5 and 8.5. Any wastewater beyond that range would need to be neutralised by the operator of the wastewater treatment system.

A study according to the test method EU B.64/OECD TG 422 must be performed in rats with oral⁹ administration of the Substance.

5. Hydrolysis as a function of pH

Hydrolysis as a function of pH is an information requirement under Annex VIII to REACH (Section 9.2.2.1.).

You have provided the following information:

1. EU Method C.7. key study with analogue substance: Dilithium7-acetamido-1-hydroxy-2-(4-((2-sulfonatooxy)ethylsulfonyl)phenylazo)naphthalene-3-sulfonate), EC 401-010-1, CAS 106027-83-2; (1985, [REDACTED]);
2. OECD 111 supporting study with analogue substance: Reaction product of 4-Amino-5-hydroxynaphthalene-2,7-disulfonic acid sodium salt and diazotized 2-[(4-Aminophenyl)sulfonyl]ethyl hydrogen sulfate, EC of the main constituent: 241-164-5, CAS of the main constituent: 17095-24-8; (1989, [REDACTED]).

We have assessed this information and identified the following issue:

According to Annex XI, Section 1.5., if the grouping concept is applied then in all cases the results to be read across must have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3), in this case OECD TG 111. Therefore, the following specification (among others) must be met:

Testing at pH values other than 4, 7, 9

- additional tests at pH values other than 4, 7 and 9 may be required for a hydrolytically unstable test substance.

Both studies provided indicate substantial hydrolytical degradation of the substances in alkaline pH. The extent of recovery rates measured in the key study differs a lot depending on the pH values tested (98% for pH 4, 70% for pH 7 but only < 1% for pH 9). This indicates significant depletion of the test substance between pH 7 and 9 and implies hydrolytical instability of the substance in alkaline pH. For that key study you have only provided one, 5-day test for pH 4, 7 and 9, in three temperatures (25, 38 and 50 °C). Based on this test you have further calculated the following hydrolysis half-lives at 25 °C: > 1 year for pH 4, 6 days for pH 7 and < 1 day for pH 9. However, you have not investigated the hydrolysis behaviour of the substance between pH 7 and 9.

In the supporting study you have performed the hydrolysis test at three pH: 4, 7 and 9. The substance is stable at pH 4 (half-life 258.5 weeks), however at pH 7 the half-life is estimated between 1 and 2 days and only 48 minutes at pH 9. The hydrolysis behaviour of the substance between pH 7 and 9 was not investigated.

Based on the above, there are critical methodological deficiencies resulting in the rejection of the study results, specifically:

⁹ ECHA Guidance R.7a, Section R.7.6.2.3.2.¹⁰ The pH of domestic wastewater is typically between 6–8 but is largely related to the alkalinity of the carriage water. In areas having soft water (alkalinity between 50 and 100 mg/L as CaCO₃), the pH of domestic wastewater is around 6.0 to 6.5. In areas having moderately hard water (alkalinity between 100 and 300 mg/L as CaCO₃) it is between 7.0 and 8.0. In areas having hard water (alkalinity higher than 300 mg/L as CaCO₃) it is between 7.5 and 9.0. Some industrial wastewaters can be quite acidic or alkaline. The optimum pH range for aerobic biodegradation lies between 6.5 and 8.5. Any wastewater beyond that range would need to be neutralised by the operator of the wastewater treatment system.

- An abrupt change of the hydrolytical behaviour is expected for the Substance between pH 7 and 9. This pH range is relevant both for the environmental assessment and for the interpretation of ecotoxicological tests. The pH of wastewater or sewage water is typically between 6–8 but can reach 8.5, implying that the substances may be hydrolysed in the wastewater or sewage water before they reach the environment¹⁰. Test guidelines for aquatic toxicity tests tolerate pH of up to 8.5 and even beyond for some of them. Therefore, investigating further the hydrolysis behaviour of the Substance between pH 7 and 8.5 is necessary for the environmental risk assessment of the Substance and for interpreting the results of the ecotoxicity tests. However, you have not considered testing hydrolysis at pH values other than 4, 7 and 9.

In your comments to the initial draft decision, you state that the hydrolysis behaviour of the Substance between pH 7 and 8.5 is irrelevant. You explain that the dye bath is adjusted to high alkalinity (pH 10-11) and temperature of 60°C, so that the substance which is not bound to the fibre at the end of the dyeing process is fully hydrolysed and therefore that no parent substance is released to the wastewater.

We note that apart from the industrial dyeing of substrates at high pH and high temperatures, other uses of the Substance are reported in your dossier (e.g. formulation, consumer uses). You have not demonstrated that complete hydrolysis of the dye occurs during those other uses. Therefore, not only the fully hydrolysed form, but potentially also the parent substance and/or mixture thereof can be present in the wastewater or sewage water. Based on that, the identity of hydrolysis product(s) and the knowledge of hydrolytical behaviour of the Substance between pH 7 and 8.5 is necessary for the environmental risk assessment of the Substance and for interpreting the results of the ecotoxicity tests.

You do not provide specific information addressing the issues identified above. Therefore, the information provided in your comments does not change the assessment outcome.

Therefore, the studies submitted in your adaptation do not provide an adequate and reliable coverage of the key parameter of the OECD TG 111.

Study design

As explained above, the hydrolysis test must be performed under slightly alkaline conditions at pH values between 7 and 8.5 and at least at pH values of 8 and 8.5.

¹⁰ The pH of domestic wastewater is typically between 6–8 but is largely related to the alkalinity of the carriage water. In areas having soft water (alkalinity between 50 and 100 mg/L as CaCO₃), the pH of domestic wastewater is around 6.0 to 6.5. In areas having moderately hard water (alkalinity between 100 and 300 mg/L as CaCO₃) it is between 7.0 and 8.0. In areas having hard water (alkalinity higher than 300 mg/L as CaCO₃) it is between 7.5 and 9.0. Some industrial wastewaters can be quite acidic or alkaline. The optimum pH range for aerobic biodegradation lies between 6.5 and 8.5. Any wastewater beyond that range would need to be neutralised by the operator of the wastewater treatment system.

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 summaries¹¹.

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 dossiers¹².

¹¹ <https://echa.europa.eu/practical-guides>

¹² <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 21 April 2021.

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

ECHA took into account your comments 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 Guidance¹³ and other supporting documentsEvaluation 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)¹⁴

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)¹⁵

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 documents¹⁶

¹³ <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

¹⁴ <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

¹⁵ https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

¹⁶ <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 their corresponding information requirements

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.