EUROPEAN CHEMICALS AGENCY

Helsinki, 13 December 2019

**Addressees**
Registrants of JS_121-03-9 listed in the last Appendix of this decision

**Date of submission for the jointly submitted dossier subject of this decision**
27 February 2012

**Registered substance subject to this decision, hereafter 'the Substance'**
Substance name: 4-nitrotoluene-2-sulphonic acid
EC number: 204-445-3
CAS number: 121-03-9

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

## DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***19 September 2022***.

**A. Requirements applicable to all the Registrants subject to Annex VII of REACH**

**1. The *in vivo* genotoxicity study also requested at C.1 below (triggered by Annex VII, Section 8.4., column 2)**

**B. Requirements applicable to all the Registrants subject to Annex VIII of REACH**

**1. The *in vivo* genotoxicity study also requested at C.1 below (triggered by Annex VIII, Section 8.4., column 2)**

**C. Requirements applicable to all the Registrants subject to Annex IX of REACH**

**1. 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 and duodenum with the Substance; duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.**

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

**3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test**

**method OECD TG 414) in a first species (rat or rabbit), oral route with the Substance;**

**Conditions to comply with the requests**

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.

To identify your legal obligations, please refer to the following:

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

Registrants are only required to share the costs of information that they must submit to fulfil the information requirements for their registration.

When a study is required under several Annexes of REACH, the reasons are provided in the corresponding appendices of this decision. 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 in accordance with Article 53 of REACH.

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

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

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

**Appeal**

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

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

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

**Appendix on general considerations**

The ECHA Guidance documents are listed in the Appendix entitled Observations and technical guidance.

**(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 read-across approaches in accordance with Annex XI, Section 1.5:

- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substances within the group (read-across approach).

A read-across hypothesis needs to be provided, establishing why a prediction for a toxicological or ecotoxicological property is reliable. This hypothesis should be based on recognition of the structural similarities and differences between the source substances and your Substance[2]. It should explain why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern.

You have provided studies for the above-mentioned standard information requirements conducted with sodium 3-nitrobenzenesulphonate (EC no. 204-857-3) and nitrobenzene (EC no. 202-716-0). You only indicated that both source substances are "structural" analogues to the Substance, without providing further justification and documentation to substantiate the structural similarity and the prediction of the properties of the Substance.

Therefore, we understand that your read-across hypothesis is only based on structural similarity between the source substances and your Substance and that this similarity is a sufficient basis for predicting the properties of your Substance.

While structural similarity is a prerequisite for applying the grouping and read-across approach, it does not necessarily lead to predictable or similar human health properties. You have not provided a well-founded hypothesis to establish a reliable prediction for a toxicological property, based on recognition of the structural similarities and differences between the source substances and your 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] ECHA Guidance R.6.

**(ii) Assessment of the use of existing data adaptation under Annex XI, Section 1.1.2.**

You seek to adapt the following standard information requirements by using existing data, in accordance with Annex XI, Section 1.1.2.:

- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

The adaptation rule in Annex XI, Section 1.1.2. imposes a number of cumulative conditions in order for existing data to be considered equivalent to data generated by the corresponding test methods referred to in Article 13(3).

One such condition is that the data must be adequate for the purpose of classification and labelling and/or risk assessment.

In your justification you simply indicate that "*testing is not scientifically necessary based on use of existing data*" with the analogue substances sodium 3-nitrobenzenesulphonate (EC no. 204-857-3) and nitrobenzene (EC no. 202-716-0).

However, the data provided is not adequate for the purpose of classification and labelling and/or risk assessment because it is data from an analogue substance and the read-across is rejected as explained in the Appendix of general considerations above.

Therefore your adaptation according to Annex XI, Section 1.1.2. is rejected.

**(iii) Assessment of exposure-based adaptation under Annex XI**

You also seek to adapt the following standard information requirements by applying the exposure-based adaptation in accordance with Annex XI, Section 3.2:

- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

Based on the arguments provided, your justification for data waiving has been evaluated under the rules set in Annex XI, Section 3.2.(a).

As stated in Annex XI, Section 3.2.(a), you may adapt the information requirement, provided you submit an adequate and scientifically-supported justification, based on a thorough and rigorous exposure assessment in accordance with Section 5 of Annex I. In addition you must fulfil all the identified criteria in paragraphs 3.2.(a), including:

(i) Results of the exposure assessment covering all relevant exposures throughout the life cycle of the Substance that demonstrate the absence of or no significant exposure in all scenarios of the manufacture and all identified uses;

Your justification indicates that the "*identified uses clearly demonstrate that there is no chronic exposure and no wide dispersive use of the substance. [...] The main part of the substance is used as an intermediate; another part is used as a monomer in polymerization processes for the production of paper dyes.*" However, we note that the Substance has also professional and consumer uses, as specified in one of the Annex VII Member dossiers that forms part of the joint submission. There is therefore exposure to the substance in at least some of the identified uses.

In your comments on the draft decision, you clarified that the Substance is only used as an intermediate or as a monomer in polymerisation processes for the production of

some organic dyes; and that the Annex VII Member, who declared [redacted] dye uses, has revised the dossier, as there are no professional and consumer uses with this Substance. Moreover, you indicated that the exposure is minimised and negligible. You stated that the only step of the life cycle where a potential exposure is possible, is the polymer manufacturing step. You also indicated that the chemical safety reports (CSR) will be updated by all the registrants to better highlight the operative conditions and the exposure potential.

ECHA acknowledges your intention to update the CSRs with the exposure information. However, based on the lack of evidence substantiating your claim currently ECHA cannot fully assess whether there is indeed "*minimised*" and "*negligible*" exposure in all uses of the Substance. Consequently, criterion (i) of Annex XI, Section 3.2. is currently not fulfilled.

(ii) DNEL derivation that takes "*full account of the increased uncertainty resulting from the omission of the information requirement*" and which is "*relevant and appropriate both to the information requirement to be omitted and for risk assessment purposes*". Moreover, footnote 1 of Annex XI, Section 3.2.(a)(ii.), specifies that "*a DNEL derived from a screening test for reproductive/developmental toxicity shall not be considered appropriate to omit a prenatal developmental toxicity study*".

As regards the DNEL derivation for the 90-day repeated dose toxicity and pre-natal developmental toxicity endpoints you used the NOAELs from the Combined repeated dose and reproduction / developmental screening study (OECD TG 422) with the Substance.

No results from test data on 90-day repeated dose toxicity and developmental toxicity are available that allow for the derivation of a DNEL for these specific hazards (90-day repeated dose toxicity and developmental toxicity)and for risk assessment purposes.

The OECD TG 422 study does not provide as much information as that provided by a 90-day repeated dose toxicity study, in particular, because in the OECD TG 422 there is a lower exposure duration and less key parameters tested than in a 90-day repeated dose toxicity study (OECD TG 408). As for the developmental toxicity endpoint, the DNEL derived from the OECD TG 422 cannot be used to omit the pre-natal developmental toxicity study (OECD TG 414), as specified under footnote 1, of Annex XI, Section 3.2.(a)(ii.).

Consequently, the criterion on the possibility to derive a DNEL from available test data appropriate both to the information requirement to be omitted and for risk assessment purposes is not fulfilled.

In your comments on the draft decision, you indicated that based on the exposure scenarios developed in the CSR, "*demonstrating no relevant exposure to the substance in any of the life cycle steps*", the second condition of the exposure-based adaptation to be fulfilled, can be "*re-considered*". However, ECHA notes that to fulfil the exposure-based adaptation according to Annex XI, Section 3.2.(a), all criteria (i) to (iii) must be met. As explained above, both criteria (i) and (ii) are currently not fulfilled.

Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 3.2.(a) and your exposure-based adaptation is rejected.

Finally, in your comments you also refer to the similarity in exposure between the use as monomer and the use as intermediate "*in the case when both are conducted in strictly controlled conditions*".

In order to fulfill Annex XI, Section 3.2.(b), you shall demonstrate and document for all relevant scenarios that throughout the life cycle, strictly controlled conditions apply, as set out in REACH Article 18(4)(a) to (f).

In your dossier you have neither indicated nor provided any documentation to substantiate that the uses are conducted under strictly controlled conditions.

Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 3.2.(b) and your exposure-based adaptation is rejected.

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

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

1. **The in vivo genotoxicity study also requested at C.1 below (triggered by Annex VII, Section 8.4., column 2)**

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

You have provided the following studies in your dossier:

i. (2010)

ii. (2010)

iii. (2010)

We have assessed this information and identified the following issue:

Under Annex VII, section 8.4, column 2, a positive result in an *in vitro* gene mutation study in bacteria raises a specific concern for gene mutation that must be addressed including, where relevant, by further studies appropriate for that specific concern.

Your dossier contains a positive result for the *in vitro* gene mutation study in bacteria, which raises the concern for gene mutation. It also contains negative results for an *in vitro* chromosomal aberration study and an *in vitro* gene mutation in mammalian cells study.

However, the concern for gene mutation raised by the positive *in vitro* gene mutation study in bacteria is not removed by the negative result in the available *in vitro* gene mutation in mammalian cells study because in the TA 100 strain (with and without metabolic activation) and the TA 98 strain (without metabolic activation), a concentration-related increase in the number of revertant colonies was observed, over the range of concentrations tested, in both trials. The positive results in TA 100 and TA 98 indicate that the Substance induces point mutations by base-pair substitutions (TA 100) and frameshift mutations (TA 98), in the *S. typhimurium*.

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

The selection of the appropriate test (TGR or comet assay) and its design are explained under Section C.1.

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

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

### 1. The in vivo genotoxicity study also requested at C.1 below (triggered by Annex VIII, Section 8.4., column 2)

Under Annex VIII to REACH, 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 to REACH.

You provided the same studies in your dossier as indicated under Section A.1.

We have assessed this information and identified the same issue as described under Section A.1.

For the same reasons as explained in Section A.1, ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified *in vitro*.

The selection of the appropriate test and its design are described under Section C.1.

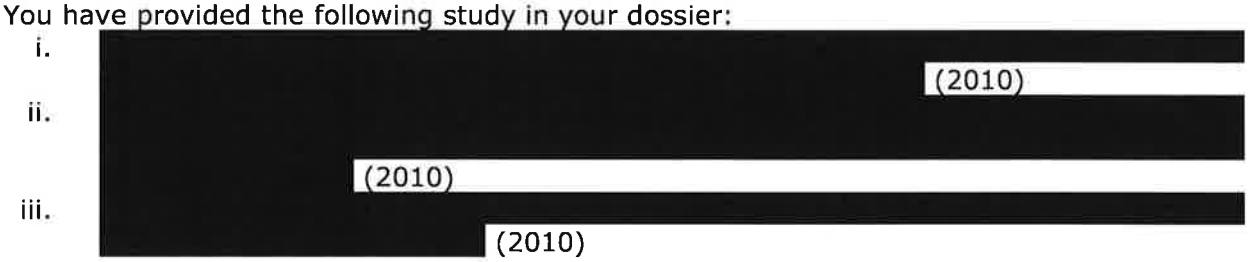
**Appendix C: Reasons for the requests to comply with Annex IX of REACH**

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes or more per year must contain, as a minimum, the information specified in Annexes VII-IX to the REACH Regulation.

## 1. *In vivo* genotoxicity study (Annex IX, Section 8.4, column 2)

Under Annex IX to REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

You have provided the following study in your dossier:




In relation to the first condition, your dossier contains positive results for the *in vitro* gene mutation study in bacteria which raise the concern for gene mutation. It also contains negative results for an *in vitro* chromosomal aberration study and an *in vitro* gene mutation in mammalian cells study. However, in relation to the second condition, no data from an *in vivo* somatic cell genotoxicity study is available in your dossier.

In your comments on the draft decision you concluded that the weak positivity in the Ames test is not relevant and that all the *in vitro* tests on genotoxicity can be considered as negative and that no further test is required. On this aspect you also refer to an EFSA scientific opinion on genotoxicity testing strategies whereby it is reported that in the case of "*inconclusive, contradictory or equivocal results from in vitro testing* [...] *it may be that further testing in vitro is appropriate to optimise any subsequent in vivo testing.*" You also refer to the ECHA Guidance Chapter R.7a where "*Expert judgement is necessary at each stage of the testing strategy to decide on the relevance of a result based on the data available for each endpoint*".

In your comments you further indicated that the positive result in the Ames study "*is related to a bacteria specific metabolism typical of nitroaromatic compounds*". You also referred to the EMA guidance on genotoxicity testing and to a publication from Kirkland *et al.* (2007)[3]; on the basis of this information you concluded that the positive Ames result with the Substance is clearly justified by the specific metabolism of bacteria and therefore this result is "*not predictive of genotoxicity in mammalian systems*". Furthermore, you provided information on three compounds (AMP397, fexinidazole, and fenitrothion) to demonstrate that the reduction of the nitro-compounds to mutagenic metabolites is due to a bacteria-specific effect. This claim was supported by the fact that, when the Ames test was performed using nitroreductase-deficient strains, the mutagenicity either decreased or completely disappeared.

We have assessed this information and identified the following issues:

[3] Kirkland D.J., Aardema M., Banduhn N., Carmichael P., Fautz R., Meunier J.R and Pfuhler S. (2007): *In vitro* approaches to develop weight of evidence (WoE) and mode of action (MoA) discussions with positive in vitro genotoxicity results. Mutagenesis 2007; 22(3):161–175

(1) In your comments you refer to the positive Ames result as "*weak*" and "*not relevant*". However, ECHA notes that the result obtained in the Ames study cannot be considered as "*inconclusive, contradictory or equivocal* ". In the TA 100 strain (with and without metabolic activation) and the TA 98 strain (without metabolic activation), a concentration-related increase in the number of revertant colonies was observed, over the range of concentrations tested, in both trials. The criteria for determing a positive result are fulfilled, according to OECD TG 471[4]. Therefore the Substance can be considered mutagenic in the Ames test.

(2) According to the ECHA Guidance R.7a, expert judgment is required at each stage of the testing strategy. Also, according to the mutagenicity testing strategy, if there is a positive Ames study at Annex VII and negative results for the Annex VIII standard information requirements, an appropriate *in vivo* mutagenicity study shall be considered (Annex VIII, Section 8.4., column 2).

(3) You did not provide evidence (including additional *in vitro* data) on the Substance to support your claim that "*the result in the Ames test is clearly justified by the specific metabolism of bacteria*". You only provide information on three aromatic nitro compounds other than the Substance. However, as indicated in the publication by Kirkland *et al.* (2007), "*not all aromatic nitro compounds are activated by nitroreduction, and care must be taken in interpreting Ames test results with this class of chemicals.*" Moreover, while additional *in vitro* data using nitroreductase-deficient Ames strains were provided for the three compounds mentioned in your comments to support your claim, such data are not available for the Substance. Finally, you commented in detail in relation to the nitro group but you did not provide any element on the possible impact of the other groups present on the aromatic ring (the methyl and the sulphonic acid) regarding the biotransformation and/or the genotoxic effect of the Substance.

With reference to point (3) above, you may consider to perform an additional Ames study with the nitroreductase-deficient strains in order to support your claim. If the result of such study would be negative in all tested nitroreductase-deficient strains, it would mean that 1) the bacterial mutagenicity was exclusively due to the nitroreduction of the aromatic nitro group and that 2) the other substituted groups of the Substance (second point mentioned above) do not impact significantly the biotransformation and/or genotoxic effect of the Substance. In such case, the *in vivo* study would not be triggered.

However, at present, the conditions set out in Annex IX, Section 8.4, column 2 are met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

According to the ECHA Guidance Chapter R.7a[5], 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.

*TGR assay:*

In case you decide to perform the TGR assay, according to the test method EU B.58/OECD TG 488, the test shall be performed in transgenic mice or rats and the test substance is usually

[4] OECD TG 471, Bacterial Reverse Mutation Test, (version of 21 July 1997), para. 35, p. 7
[5] ECHA Guidance Chapter R.7a, Section R.7.7.6.3

administered orally.

According to the test method EU B.58/OECD TG 488, the test shall 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 shall be stored (at or below −70 °C) until the analysis of liver and glandular stomach is completed; the duodenum shall then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

*Comet assay:*

In case you decide to perform the comet assay according to the test method OECD TG 489, the test shall 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 test method OECD TG 489, the test shall 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.

*Germ cells:*

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

Therefore, in case you decide to perform the comet assay, you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[6]) in addition to the other aforementioned tissues, 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 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 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 °C). Following the generation and analysis of data on somatic cells, in accordance to Annex IX, Section 8.4., column 2, you

[6] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

should consider analysing 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.

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

A Sub-chronic toxicity study (90-day) is a standard information requirement in Annex IX to REACH.

You have provided a key study and a non-guideline supporting study for this endpoint in your dossier:

i. [redacted] (2010); and

You refer to the supporting study which is conducted with an analogue substance as existing data which fulfils the criteria of Annex XI section 1.1.2. You also refer to both key and supporting studies as part of a weight of evidence adaptation in accordance with Annex XI, Section 1.2. Finally, you provide an exposure based adaptation in accordance with Annex XI, Section 3.2.(a).

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

1) Key study

To be considered compliant and enable concluding whether the Substance has dangerous properties and supports the determination of the No-Observed Adverse Effect Level (NOAEL), a study has to meet the requirements of OECD TG 408. The following key parameters of this test guideline include, among others, dosing of the Substance daily for a period of 90 days until the scheduled termination of the study.

However, the study i. does not have the exposure duration of 90 days as required in OECD TG 408, because the exposure duration in the screening study was 41 to 48 days (for females) and 49 to 50 days (for males).

Therefore, the study is not sufficient to meet this information requirement.

2) Invalid adaptation under Annex XI, Section 1.5. (read-across)

As explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.5. is rejected.

3) Supporting study and adaptation according to Annex XI, Section 1.1.2

As explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.1.2. is rejected because it is data from an analogue substance and the read-across is rejected as also explained in the Appendix of general considerations.

In case you wish to improve your read-across justifcation, ECHA has also assessed the data against the other conditions of Annex XI, Section 1.1.2.

The adaptation rule in Annex XI, Section 1.1.2 imposes a number of cumulative conditions for an adaptation to be valid. In particular the data must provide adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3); in this case the appropriate test method is OECD TG 408 and some of the key parameters of this method include that

- the highest dose level should aim to induce some systemic toxicity, but not death or severe suffering;
- At least 10 female and 10 male animals should be used at each dose level (including control group).

However, the study has been performed on a hair dye formulation containing only 2.25% of the analogue substance. The top dose in this study was 97.5 mg/kg/bw of the formulation, which equates to an exposure of 2.2 mg/kg/bw of the analogue substance. The dose level tested is considered too low, as it was not not inducing any systemic toxicity and it was not proven that the aim was to induce some systemic toxicity. Therefore, it does not allow for conclusions to be drawn on the potential effects of the analogue substance in a 90 day repeated dose toxicity study. Additionally, the study was conducted with less than 10 animals per sex per test dose group (only 6 animals per sex per dose group used). The statistical power of the information provided is not sufficient because it does not fulfil the criterion of 20 animals (10 males + 10 females) for each test group set in OECD TG 408. Therefore, the sensitivity of this study is much lower than that of a 90-day study.

Therefore your adaptation according to Annex XI, Section 1.1.2. is rejected.

4) Invalid adaptations under Annex XI, Section 3 (exposure based adaptation)

As explained in the Appendix on general considerations your adaptation according to Annex XI, Section 3.2. is rejected.

5) Invalid adaptations under Annex XI, Section 1.2 (weight of evidence)

You also seek to adapt the standard information requirement according to Annex XI, Section 1.2. Weight of evidence.

Annex XI, Section 1.2 states that there may be sufficient weight of evidence (WoE) 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 WoE adaptation involves an assessment of the relative values/weights of different pieces of the available information which is defined by e.g. the reliability of the data, consistency of results/data, nature and severity of effects, and relevance of the information for the given regulatory endpoint. Subsequently, the lines of evidence should be integrated considering their relative values or weights in order to draw a conclusion. Adequate and reliable documentation shall be provided to describe your WoE approach, the assessment of relative weights of individual piece of information and the subsequent conclusions drawn.

In order to allow a conclusion on the repeated dose toxicity of the Substance in a WoE adaptation, the justification must cover the key parameters foreseen to be investigated in a sub-chronic toxicity study (90-day).

You have justified the WoE adaptation by referring to studies i. and ii. as listed above and concluding that, there is sufficient WoE that the Substance does not have a dangerous

property and that additional testing is considered to be of very low priority.

As already explained under issues 1) and 2) above, where studies i. and ii. are individually addressed, certain key parameters of the OECD TG 408 are not met in each of these studies.

More specifically, in study i. the exposure duration is not sufficient. Therefore, the provided piece of information gives only a very low weight for your WoE adaptation.

Regarding study ii., the study uses low doses and less animals der dose group than that required by the OECD TG 408. In addition the study is conducted on an analogue substance and the read across has been rejected as explained in the Appendix on general considerations. Consequently, this study has no weight in your WoE adaptation.

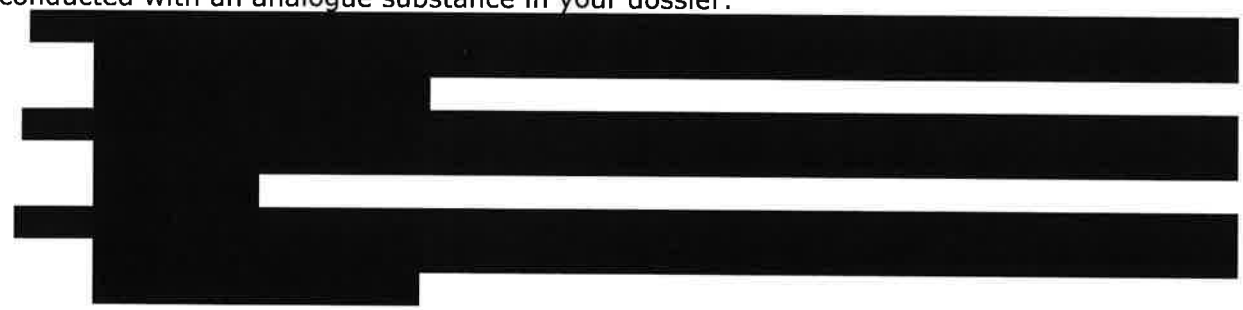
Based on the assessment above, it is not possible to conclude, on the basis of any source of information alone or considered together, and taking into account your justification for the weight of evidence adaptation whether your Substance has or has not the particular dangerous property foreseen to be investigated in an OECD TG 408 study. Your adaptation is rejected and the information requirement is not fulfilled.

Following the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity because the Substance is a solid, however is used in a non-solid form and formulated only in wet processes. The sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and with oral administration of the Substance, because the available Combined repeated dose and reproduction / developmental screening study with the Substance (OECD TG 422) performed via the oral route (gavage administration) indicates a concern for toxicity in various organs, including liver, kidney, spleen and reproductive organs, that requires further information on repeated dose toxicity by the oral route. Moreover, the Substance has a very low vapour pressure (0.0001 Pa) and high water solubility (667 g/l at 23°C).

### 3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

You have provided a key study conducted with the Substance and two supporting studies conducted with an analogue substance in your dossier:



You refer to the supporting studies which are conducted with an analogue substance as existing data which fulfil the criteria of Annex XI section 1.1.2. You also provide an exposure based adaptation in accordance with Annex XI, Section 3.2.(a).

We have assessed this information and identified the following issues:

1) Key study

In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, information provided has to meet the requirements of OECD TG 414 in one species. OECD TG 414 has an number of key parameters, including:

- 20 female animals with implantation sites for each test and control group,
- examination of the foetuses for sex and body weight/external, skeletal and soft tissue alterations (variations and malformations)/number of resorptions and or live foetuses/ measurement of anogenital distance in live rodent foetuses.

The key study is an OECD TG 422 study and does not cover the key parameters of the OECD TG 414. More specifically, structural malformations and variations are not investigated. Also, the study was conducted with only 10 to 12 pregnant females for each test group. The statistical power of the information provided is not sufficient because it does not fulfil the criterion of 20 pregnant females for each test group.

Therefore, the study is not sufficient to meet this information requirement.

2) Supporting studies and adaptation according to Annex XI, Section 1.1.2 (existing data).

As explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.1.2. is rejected.

3) Invalid adaptation under Annex XI, Section 1.5. (read-across)

As explained in the Appendix on general considerations your adaptation according to Annex XI, Section 1.5. is rejected.

4) Invalid adaptations under Annex XI, Section 3 (exposure based adaptation)

As explained in the Appendix on general considerations your adaptation according to Annex XI, Section 3.2. is rejected.

Therefore, the information requirement is not fulfilled.

A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species with oral[7] administration of the Substance.

[7] ECHA Guidance R.7a, Section R.7.6.2.3.2.

## Appendix D Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of the REACH Regulation.

The compliance check was initiated on 4 March 2019.

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

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

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: Observations and technical guidance

1. The information requirement under Section 8.7.3. of Annex IX to REACH (Extended one-generation reproductive toxicity study, EOGRTS) is not addressed in this decision, because the information from the Sub-chronic toxicity study (90-day), requested in the present this decision, is relevant for the design of the EOGRTS.

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

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

4. Test guidelines, GLP requirements and reporting

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

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

   Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries[8]'.

5. Test material

   Selection of the test material(s)

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

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

   Technical reporting of the test material

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

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

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[3].

6. List of references of the ECHA Guidance documents[9]

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

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

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

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.

[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

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

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