~~Confidential~~

Helsinki, 24 June 2021

Addressees
Registrants of JS_Monoazored_PO5 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
19 June 2019

Registered substance subject to this decision ("the Substance")
Substance name: 1-[(2,4-dinitrophenyl)azo]-2-naphthol
EC number: 222-429-4
CAS number: 3468-63-1

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 in A.1 to A.3 and B.5 below by ***31 March 2022*** and all other information listed below by ***1 June 2023***.

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

We note that the Substance has been notified as a nanoform under the French nano-particulate substances reporting system.[1] This indicates that the Substance is manufactured or imported in the European Union in nanoforms, possibly by any addressee of the present decision. However, the REACH Regulation (as amended by Regulation Commission Regulation (EU) 2018/1881) sets out explicit information requirements for nanoforms of substances. Manufacturers and importers of nanoforms must have fulfilled these specific information requirements by 1st January 2020. As far as the registration dossier currently submitted on the Substance does not cover any nanoform, the incompliances identified in the present decision relate only to information required on non-nanoforms.

Based on the above, the requested information must be generated using exclusively non-nanoforms of the Substance.

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

1. Water solubility (Annex VII, Section 7.7.; test method: EU A.6./OECD TG 105)

2. Partition coefficient n-octanol/water (Annex VII, Section 7.8.; test method: EU A.8 or OECD TG 117 or OECD TG 123)

3. Ready biodegrability (Annex VII, Section 9.2.1.1.; test method: OECD TG 301B/C/D/F or OECD TG 310)

4. Same *In vitro* cytogenicity study in mammalian cells (test method: OECD TG 473) or *In vitro* micronucleus study (test method: OECD TG 487), as requested in B.1 below

[1] "*Dispositif de déclaration des substances à l'état nanoparticulaire*", Decree 2012-232 of French *Conseil d'Etat* of 17 february 2012.

5. Same *in vivo* genotoxicity study, as requested in B.2. below

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 of the *in vitro* study requested in B.1** (*in vitro* cytogenicity study in mammalian cells) are **negative**:

   In vivo mammalian alkaline comet assay (test method OECD TG 489) in rats, oral route, on the tissues: liver, glandular stomach and duodenum

   OR

   Transgenic rodent somatic and germ cell gene mutation assay (test method: OECD TG 488 from 2020[2]) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; 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 *in vitro* study requested in B.1** (*in vitro* cytogenicity study in mammalian cells) are **positive**:

   In vivo mammalian alkaline comet assay (test method OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. 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.64/OECD TG 422) by oral route, in rats

5. Adsorption/ desorption screening (Annex VIII, Section 9.3.1.; test method: test method: OECD TG 121 or OECD TG 106)

Reasons for the request(s) are explained in the following 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

[2] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66.

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

Appeal

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

Failure to comply

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

Authorised[3] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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

Appendix on Reasons common to several requests

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

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

- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Ready biodegradability (Annex VII, Section 9.2.1.1.)

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

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category (addressed under 'Scope of the grouping'). Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (addressed under 'Assessment of prediction(s)').

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

A. Scope of the grouping

In your registration dossier you have formed a group (category) of 'Monoazo Red Pigments'. You have provided a read-across justification document in Section 1 of your CSR.

For the purpose of this decision, the following abbreviations are used for the group members:

1) "PR3": C.I. PIGMENT RED 3, i.e. 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC No. 219-372-2);
2) "PR4": C.I. PIGMENT RED 4, i.e. 1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol (EC No. 220-562-2);
3) "the Substance".

You provide the following reasoning for the grouping the substances: *"The pigments grouped in this category* [...] *contain a substituted phenyl moiety, an azo moiety, and a 2-hydroxynaphthalene (β-naphthol)* [...]*".*

You define the structural basis for the grouping as all substances with the above structural groups but with *"different identity of the substituents of the phenyl ring"*. ECHA understands that this is the applicability domain of the grouping and will assess your predictions on this basis.

B. Predictions for properties

a. Prediction for environmental fate properties

You have provided the following reasoning for the prediction of environmental fate properties:

- *"All members of this category are solids, which decompose at high temperatures. The solubility of these red and orange pigments in water and n-octanol is limited, < 18 mg/L, resulting in a low partition coefficient in n-octanol/water (log Pow < 3.7)* [...]";

- "*All category members tested showed very limited biodegradability, which is assumed to be due to their unavailability for microorganisms*";
- "*Monoazo Red Pigments do not hydrolyse in aqueous solutions, i.e. no hazardous substances are liberated from these pigments*";
- You conclude that "*these data indicate that the presence, number and identity of substituents on the phenyl ring do not influence the physical-chemical, ecotoxicological and toxicological behaviour of the pigments in a significant way*".

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

You intend to predict the ready biodegradability property of the Substance from information obtained from the following source substance: PR3, i.e. 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC number 219-372-2).

ECHA notes the following issue with regards to prediction of environmental fate properties:

Supporting information

Annex XI, Section 1.5 of the REACH Regulation states that "[…] *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 other category members.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar category members cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the category members is necessary to confirm that both substances cause the same type of effects.

As you have only provided information on ready biodegradability for the source substance PR3, you have not demonstrated that "*all category member show very limited biodegradability*". Furthermore, similar physicochemical properties and the low likelihood for hydrolysis due to low solubility is not sufficient to demonstrate similar (low) biodegradation potential. Therefore, the data set reported in the technical dossier does not include relevant, reliable and adequate information for the category members to support your read-across hypothesis.

In the absence of such information, you have not established that the category members are likely to have similar properties. Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

b. Prediction for toxicological properties

You have provided the following reasoning for the prediction of toxicological properties:

- As explained above, you state that the category members have similar physicochemical properties;
- "*None of the category members showed a toxic effect after single oral or inhalational exposure, no skin or eye irritation, no skin sensitizing effect, and no mutagenic*

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

properties in any study (OECD 473, 476, 482) except in Ames assays (OECD 471)";
- "*the bioavailability of the Monoazo Red Pigments after oral, dermal or inhalative exposure is low to negligible* [which] *is probably the reason for the absence of any relevant mammalian toxicity*".

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

You intend to predict screening for reproductive/developmental toxicity of the Substance from information obtained from the following source substance: PR3, i.e. 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC number 219-372-2).

ECHA notes the following issues with regards to prediction of environmental fate properties:

1. *Read-across hypothesis*

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 substance(s) 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 substance(s) and your Substance[5]. 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.

Your read-across hypothesis is that the toxicological similarity between the category members in one or multiple endpoints is a sufficient basis for predicting the properties of the Substance for other endpoints.

However, toxicological similarity in one or multiple endpoints does not necessarily lead to predictable or similar human health properties in other endpoints. You have not provided a well-founded hypothesis to establish a reliable prediction for a toxicological, based on recognition of the structural similarities and differences between the category members.

2. *Supporting information*

Annex XI, Section 1.5 of the REACH Regulation states that "[…] *toxicological properties 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*"[6]. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on other category members.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar category members cause the same type of effect(s). In this context,

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

relevant, reliable and adequate information allowing to compare the properties of the category members is necessary to confirm that both substances cause the same type of effects.

You consider that low to negligible bioavailability of the Monoazo Red Pigments is expected to lead to the absence of any relevant mammalian toxicity. However, you have not provided any experimental evidence to support that lack of bioavailability of the substance, including for instance toxicokinetic data on all category members. Furthermore, you have not provided any supporting information on the category members to demonstrate that they may have similar reproductive/developmental toxicity properties.

In the absence of such information, you have not established that the category members are likely to have similar properties. Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

C. Conclusions on the grouping of substances and read-across approach

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

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

1. Water solubility

Water solubility is an information requirement under Annex VII to REACH (Section 7.7).

You have provided the following information:

- Study similar to OECD TG 105, key study, [redacted] (2006).

We have assessed this information and identified the following issue:

To fulfil the information requirement, a study must comply with the OECD TG 105 or the EU Method A.6 (Article 13(3) of REACH). Therefore, the following specifications must be met:

- the shake-flask method is applicable to test material with a water solubility ≥ 10 mg/L;
- solids are pulverized before testing;
- the test is conducted with a loading of about five times the quantity required to saturate a given volume of water;
- three flasks are included which are shaken/stirred for 24, 48 and 72 hours, respectively;
- after shaking/stirring, each flask is equilibrated for 24 hours at 20°C;
- the results are considered acceptable, if the results of the flasks shaken for 48 and 72 hours differ by ≤ 15%. If the results shows a tendency of higher solubility with longer shaking/stirring period, the test is repeated with longer equilibration times;
- a reliable analytical method is available.

Your registration dossier provides a study showing the following:

- the water solubility was determined to be 6.3 µg/L, hence below 10 mg/L;
- the fact that the test material was pulverized or not before testing is not reported;
- about 5 mg of the test sample were suspended in 30 mL water in a sample flask;
- triplicate test samples were shaken for two hours at 30°C (+/- 2°C) and then at ambient temperature (c.a. 22-23°C) for 70 hours;
- the test material concentration was determined UV-VIS. The calibration curve was produced using chloroform as solvent with a 10 mm cuvette. The values obtained for the calibration curve are not reported (only a graph is provided). The measured absorbance for the test material ranged from 0.00327 to 0.00749 (measured at 481 nm in water with a 100 mm cuvette).

Based on the above, the shake-flask method described in OECD TG 105 is not applicable to the Substance as its solubility is estimated to be well below 10 mg/L. Furthermore, the test design, the loading rate and the sample preparation method are not compliant with the guideline requirements. Finally, the analytical method used in this study did not allow providing a reliable estimate of dissolved concentration. The measured absorbance values in the test samples are more than an order of magnitude below the absorbance value of the lowest calibration point. Considering the inherent uncertainty related to the measurement of low absorbance values and the fact that the calibration curve and test samples use different solvents (i.e. chloroform versus water, which have different λmax), the reliability of the reported analytical method is not demonstrated.

On the basis of the above, the information requirement is not fulfilled.

Study design

Considering the properties of the Substance (solubility < 10 mg/L), the column elution described in EU A.6/OECD TG 105 is the most appropriate method to fulfil the information requirement for the Substance.

2. Partition coefficient n-octanol/water

Partition coefficient in n-octanol/water is an information requirement under Annex VII to REACH (Section 7.8).

You have provided the following information:

- Study similar to OECD TG 107, [redacted] (2006).

We have assessed this information and identified the following issues:

A. To fulfil the information requirement, a study must comply with the OECD TG 107 or OECD TG 117 or OECD TG 123 or the EU Method A.8 (Article 13(3) of REACH). These test guidelines describe three methods (the shake flask method, the HPLC method and the slow-stirring method) for conducting the determining the partition coefficient between water and n-octanol (Log Kow). The EU test method A.8 specifies that the method selection must be based on the properties of the substance and on a preliminary determination of Log Kow using the individual solubilities of the test material in water and n-octanol. This preliminary estimate is considered sufficient only if none of the recommended method are technically feasible due to specific substance properties (*e.g.* surface active substances).

   Your registration dossier provides a study claimed similar to OECD TG 107. However, the robust study summary reports that the study was conducted according to the ETAD method where log Kow is determined using the individual solubilities of the test material in water and n-octanol. You have not provided any justification as to why none of the methods listed above are technically feasible.

B. To provide an acceptable determination of the partition coefficient using individual solubilities in water and n-octanol, the calculation must be based on reliable individual solubilities estimates.

   You used the information discussed under Section A.1 as the water solubility estimated used in the calculation. You report that the n-octanol solubility estimate was determined using a similar method.

   As explained under Section A.1, the information provided in your registration does not fulfil the information requirement. Furthermore, as a similar approach was used to determine n-octanol solubility, similar issue identified under Section A.1 also apply to the determination of n-octanol solubility. Hence, the log Kow value reported in your registration dossier is not reliable.

On the basis of the above, the information requirement is not fulfilled.

Study design

Considering the properties of the Substance (sparingly soluble particles), the Partition Coefficient (n-octanol/water), HPLC Method (test method: OECD TG 117) or alternatively the

Partition Coefficient (1-Octanol/Water): Slow-Stirring Method (test method: OECD TG 123) are the most appropriate method to fulfil the information requirement for the Substance.

3. Ready biodegradability

Ready biodegradability is an information requirement under Annex VII to REACH (Section 9.2.1.1.).

You have provided an adaptation under Annex XI, Section 1.5.

However, for the reasons explained under Appendix on Reasons common to several requests, your adaptation is rejected.

On the basis of the above, the information requirement is not fulfilled.

4. In vitro cytogenicity study in mammalian cells or In vitro micronucleus 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.7a[7] further specifies that "*REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII*." This is for the reason that the *in vitro* cytogenicity test under Section 8.4.2 will allow to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy. The obtained *in vitro* data will inform on the genotoxic concern(s) associated with the substance and help identify the most adequate follow-up *in vivo* study (same *in vivo* study requested under A.5. and B.2).

For the assessment, selection and specifications of the study to be performed, see section B.1.

5. In vivo genetic toxicity 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.*7a*[8] 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 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 which raise the concern for gene mutation.

ECHA considers that an appropriate *in vivo* follow up genetic toxicity study is necessary to address the concern identified *in vitro*. For the assessment, selection and specifications of the study to be performed, see section B.2.

[7] ECHA Guidance R.7a, section R.7.7.6.3, p.570.
[8] ECHA Guidance R.7a, section R.7.7.6.3, p.570.

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 an information requirement under Annex VIII to REACH (Section 8.4.2).

You have provided the following information:

i. *In vitro* chromosome aberration study in mammalian cells with the Substance ([redacted], 1989), according to OECD TG 473.

You also provided the following *in vivo* studies in your dossier:

ii. Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* with the Substance ([redacted], 2013), according to OECD TG 486.

iii. *In vivo* mammalian bone marrow chromosome aberration test with the Substance ([redacted], 1990), according to OECD TG 475.

Although you do not explicitly claim an adaptation, ECHA notes that the studies i. and iii. are not performed according to Good Laboratory Practice (GLP) as required under Article 13(4). We understand that you may have submitted these studies as an adaptation according to Annex XI, Section 1.1.2.

With the submission of the *in vivo* studies ii. and iii., we understand that you have attempted to adapt this information requirement under Section 8.4.2., Column 2, first indent, Annex VIII to REACH.

We have assessed this information and identified the following issues:

A. Non GLP studies and adaptation under section 1.1.2 of Annex XI

An adaptation according to Annex XI, Section 1.1.2 enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods.

The adaptation rule in Annex XI, Section 1.1.2. imposes a number of cumulative conditions for an adaptation to be valid, in particular:

1. Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test method (OECD TG 473/487);
2. Adequate and reliable documentation of the study is provided. More specifically, Article 10(a)(vii) and Article 3(28) require documentation studies to be reported in the form of a robust study summary.

However, we note that there is:

1. No adequate and reliable coverage of the key parameters

   a. Concerning study i. the corresponding test method is OECD TG 473 or 487. One of the key parameters of OECD TG 473 includes the performance of three experimental conditions: a short treatment with and without metabolic activation and a long treatment without metabolic activation.

      However, the reported data for the study does not include the long treatment without the metabolic activation. Therefore, study i. does not provide adequate

and reliable coverage of the key parameters foreseen to be investigated under OECD TG 473.

b. Concerning study iii. the corresponding test method is OECD TG 475. The key parameters of OECD TG 475 include:
   - The study must include a minimum of three doses/groups of treated animals, as well as a negative control group and a positive control group.
   - At least 200 metaphases must be analysed for each animal for structural chromosomal aberrations including and excluding gaps.

   However, the reported data for the study you have provided did not include:
   - the appropriate number of doses.
   - the appropriate number of metaphase cells analysed per animal.

2. No adequate and reliable documentation

   a. Concerning study i. you have not reported information, such as:
      i. test conditions (use of solvent/vehicle, number of metaphases analysed, positive and negative control substances, final concentrations for each conditions of treatment, etc.); and
      ii. results (number of cells scored, concurrent negative (solvent) and positive control data (concentrations and solvents), etc.).
   b. Concerning study iii. you have not reported information, such as results (data on the mitotic index and the mean number of cells with aberrations per group for each group of animals).

Therefore, the studies i. and iii. are not adequate and an adaptation under section 1.1.2. cannot be accepted.

B. Adaptation under column 2 of Section 8.4.2 of Annex VIII to REACH

Under Section 8.4.2., Column 2, first indent, Annex VIII to REACH, the study may be omitted if adequate data from an *in vivo* cytogenicity *test* are available. The *in vivo* study must be either a micronucleus test or a chromosomal aberration test, performed according to OECD TG 474 or 475, respectively[9].

You have provided an Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (OECD TG 486).

This test is not a micronucleus test or a chromosomal aberration test. Furthermore, as already explained above under issue A., the study according to OECD TG 475 (study iii. above) provided in your dossier does not meet the information requirement.

Therefore, the requirements of Section 8.4.2., Column 2, first indent, Annex VIII to REACH are not met.

On the basis of the above, the information requirement is not fulfilled.

Study design

To fulfil the information requirement for the Substance, either *in vitro* cytogenicity study in mammalian cells (test method OECD TG 473) or *in vitro* micronucleus study (test method

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

OECD TG 487) are considered suitable.

2. In vivo genetic toxicity 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 which raise the concern for gene mutation.

You have provided the following *in vivo* studies:

i. Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* with the Substance ([redacted], 2013), according to OECD TG 486.
ii. *In vivo* mammalian bone marrow chromosome aberration test with the Substance ([redacted], 1990), according to OECD TG 475.

We have assessed this information and identified the following issues:

i. Suitable OECD TG

According to the ECHA Guidance Chapter R.7a[10], the transgenic rodent somatic and germ cell gene mutation assay ("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.

However, you provided a study according to OECD TG 486.

This test is neither a transgenic rodent somatic and germ cell mutation assay nor a comet assay.

Therefore, the provided *in vivo* test is not adequate.

ii. Specific concern raised by in vitro positive results

In order to be appropriate, 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 *in vivo* study ii. is not addressing the gene mutation concern raised by the *in vitro* data. Therefore, this *in vivo* test is not appropriate.

Moreover, as explained under section 1 of Appendix B, this study is not reported, as required, in the form of a robust study summary and does not cover the key parameters foreseen to be investigated under OECD TG 475.

On the basis of the above, an appropriate *in vivo* follow-up mutagenicity study is necessary to address the concern identified *in vitro*.

i. Test selection

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

[10] ECHA Guidance Chapter R.7a, Section R.7.7.6.3

However, this decision also requests an *in vitro* test (see Section B.1), which could raise a concern for chromosomal aberration in case of positive results.

In case there is also a concern for chromosomal aberration, you must combine the comet assay and the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) into a single study. The MN test is a mutagenicity test that provides evidence on *in vivo* chromosomal mutagenicity, as the study detects both structural and numerical chromosomal aberrations. The combined study can help reduce the number of tests performed and the number of animals used while addressing both chromosomal aberration and gene mutation.

Therefore, you must wait for the result of the *in vitro* test requested under B.1 and, depending on the result, to conduct either a) Comet assay or TGR, if the test results of request B.1 are negative; or b) Comet assay combined with MN test if the test results of request B.1 are positive. The deadline set in this decision allows for sequential testing.

ii. Study design

*a) Comet assay or TGR assay (if the test results of request B.1 are **negative**)*

Comet assay:
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 test method 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.

TGR assay:
According to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally.

Based on the recent update[11] of OECD TG 488, 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. This updated version provides for a transitional period for the new version. However, ECHA is aware that testing according to the updated OECD TG is already available from CROs and the new study design would provide meaningful germ cell data, so this decision requires the application of the new version.

According to the test method 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

[11] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66

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 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

Germ cells
In case you decide to perform the comet assay, you may consider to collect the male gonadal cells 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, 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 ºC). 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.

*b) Comet assay combined with MN test (if the test results of request B.1 are **positive**)*

According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combined test (OECD TG 489 and OECD TG 474) must be performed in rats. Having considered the anticipated routes of human exposure and the need for 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 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.

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 the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al*. 2011[12]).

Germ cells
You may consider to collect the male gonadal cells 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

[12] 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

analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal 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 (28 days)

A short-term repeated dose toxicity study (28 days) is an information requirement under Annex VIII to REACH (Section 8.6.1.).

You have provided the following information:

i. study similar to OECD TG 407, [redacted] (1973);
ii. study similar to OECD TG 408, [redacted] (1959);

For the inhalation and dermal route, you have provided justifications to support that these exposure routes are not the most appropriate.

With the submission of study ii, we understand that you aimed to rely on an adaptation under Section 8.6., Column 2, first indent, Annex VIII to REACH.

We have assessed this information and identified the following issues:

A. To fulfil the information requirement, a study must comply with OECD TG 407. Therefore, the following sepcifications must be met:
   - animals in a satellite group scheduled for follow-up observations are kept for at least 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.
   - animals that die or are euthanised during the test are necropsied;
   - clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, are performed. Investigations of plasma or serum include sodium, potassium, glucose, total cholesterol, urea, creatinine, total protein and albumin, at least two enzymes indicative of hepatocellular effects (such as alanin aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl trans-peptidase and glutamate dehydrogenase), and bile acids;
   - in the fourth exposure week sensory reactivity to stimuli are conducted;
   - full histopathology is carried out on the preserved organs and tissues of all animals in the control and high dose groups. Histopathological examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group. Tissues to be examined include all gross lesions, brain, spinal cord, eye, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs, gonads, accessory sex organs, vagina, urinary bladder, lymph nodes, peripheral nerve, skeletal muscle and bone, with bone marrow;

   Your registration dossier provides a study claimed similar to OECD TG 407. However, you do not report that a satellite group was included to evaluate the reversibility of effects. There is no reporting of necropsy of animals that died in the course fo the study. No reporting of clinical biochemistry determinations or examination of sensory reactivity to stimuli is reported and all relevant tissues specified in OECD TG 407 were not investigated. Finally, this study does not provide a comprehensive description of the results which does not allow an independent assessment of the reliability of the study conclusions. Therefore, this study does not meet the information requirement.

B. Under Section 8.6., Column 2, first indent, Annex VIII to REACH, the study may be omitted if reliable sub-chronic (90 days) or chronic toxicity study is available, provided an

appropriate species, dosage, solvent and route of exposure were used. For a sub-chronic study via the oral route, a study must comply with OECD TG 408. Therefore, the following requirements must be met:

- at least 20 animal (ten female and ten male) are used at each dose level;
- at least three dose levels and a concurrent control are used unless a limit test at 1000 mg/kg bw/d produces no observed adverse effects;
- the animals are dosed with the test chemical daily seven days each week for at least 90 days. Any other dosing regime needs to be justified;
- sensory reactivity to stimuli are investigated towards the end of the exposure period and not earlier than week 11;
- body weight and food/water consumption are determined at least weekly;
- haematological and clinical biochemistry investigations are conducted;
- all animals in the study are subject to a full, detailed gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, testes, epididymitis, prostate + seminal vesicles with coagulating glands, uterus, ovaries, thymus, spleen, brain, and heart;
- full histopathology is carried out on the preserved organs and tissues of all animals in the control and high dose groups;

Your registration dossier provides a study claimed similar to OECD TG 408 which shows the following:

- a single dose of 100 mg/kg bw/day was studies;
- ten animals were used;
- the test substance was administered 65 times in 97 days. Not justification for the dosing regime is provided;
- it is stated that "*the autopsy showed neither macroscopically nor in the histological examination of the organs any pathological findings*". However, no information on body weight and food/water consumption determinations, haemathological and clinical biochemistry investigations, gross necropsy and histopathology of relevant organs are provided.

The provided study does not comply with OECD TG 408 because a single low dose was tested, the number of animals is too low, test animals were not dosed daily and no justification is provided and the investigated parameters does not provide a comprehensive coverage of the parameters that must be investigated under this test guideline. Therefore, this study is not regarded as a reliable sub-chronic (90 days) toxicity study. If you had intended to rely on an adaptation under column 2 of Section 8.6., Column 2, first indent, Annex VIII to REACH, this adaptation is rejected.

C. To comply with this information requirement, the test material in a study must be representative for the Substance (Article 10 and Recital 19 of REACH; ECHA Guidance R.4.1).

For study i. and ii. above, you have identified the test material as "*Hansaorange RN 01*", without further information, including composition and the presence of impurities.

In the absence of composition information on the test material, the identity of the test material and its impurities cannot be assessed and you have not demonstrated that the test material is representative for the Substance.

On the basis of the above, the information requirement is not fulfilled.

Study design

Further information on the study design are provided under Section B.4. below.

4. Screening for reproductive/developmental toxicity

A screening for reproductive/developmental toxicity study is an information requirement under Annex VIII to REACH (Section 8.7.1.), 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.

You have provided an adaptation under Annex XI, Section 1.5.

However, for the reasons explained under Appendix on Reasons common to several requests, your adaptation is rejected.

On the basis of the above, the information requirement is not fulfilled.

Study design

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407) (as explained above under section B.3), 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.[13]

The oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction (ECHA Guidance R.7.6.2.3.2). Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route. Therefore, a study according to the test method EU B.64/OECD TG 422 must be performed in rats with oral[14] administration of the Substance.

5. Adsorption/ desorption screening

Adsorption/desorption screening is an information requirement under Annex VIII to REACH (Section 9.3.1).

You have adapted this information requirement with the following justification: "*The study does not need to be conducted because the substance has a low octanol water partition coefficient and the adsorption potential of this substance is related to this parameter. In accordance with Column 2 adaptation statement of REACH Annex VIII and IX, adsorption/desorption screening and further studies on adsorption/desorption, information requirements 9.3.1 and 9.3.3, may be omitted since the log Kow value for the test substance is <3.0 (CSR sections 1.3 and 4.2.1) and has low potential for adsorption*".

We have assessed this information and identified the following issue:

[13] 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)
[14] ECHA Guidance R.7a, Section R.7.6.2.3.2.

Under Section 9.3.1., Column 2, first indent, Annex VIII to REACH, the study may be omitted if based on the physicochemical properties the substance can be expected to have a low potential for adsorption (e.g. the substance has a low octanol water partition coefficient).

However, for the reasons explained under Section A.2 the information requirement for the partition coefficient in n-octanol/water (Section 7.8, Annex VII of REACH) is not fulfilled and your adaptation is rejected.

On the basis of the above, the information requirement is not fulfilled.

Study design

Considering the properties of the Substance (sparingly soluble particles), the Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC) (test method: OECD TG 121) or alternatively the Adsorption/Desorption Using a Batch Equilibrium Method (test method: OECD TG 106) are the most appropriate method to fulfil the information requirement for the Substance.

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

A. Test methods, GLP requirements and reporting

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

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

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[15].

B. Test material

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

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

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

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

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[16].

[15] https://echa.europa.eu/practical-guides
[16] https://echa.europa.eu/manuals

Appendix D: Procedure

The Substance is listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2019/2020.

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 24 March 2020.

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

Comments related to registration issues were provided by one addressee. These were addressed through a separate communication.

ECHA did not receive any comments within the commenting period on the requests listed in the decision.

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-74 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix E: List of references - ECHA Guidance[17] and other supporting documents

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

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

Read-across assessment framework (RAAF, March 2017)[18]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[18]

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.

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

OECD Guidance documents[19]
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.

[19] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

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

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

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