ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 29 March 2019

Addressee: [redacted]

Decision number: CCH-D-2114461479-37-01/F
Substance name: 1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol
EC number: 220-562-2
CAS number: 2814-77-9
Registration number: [redacted]
Submission number: [redacted]
Submission date: 03/05/2017
Registered tonnage band: 10-100

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6.);**

2, 3. **Combined repeated dose toxicity study with the reproduction/developmental toxicity screening study (Annex VIII, Section 8.6.1. and Section 8.7.1.; test method: OECD TG 422) in rats, oral route with the registered substance;**

4. **In vivo mammalian alkaline comet assay (Annex VIII, 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 registered substance**

   or

   Transgenic rodent somatic and germ cell gene mutation assays (Annex VIII, 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 and glandular stomach with the registered substance; germ cells and 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. The test material used should be freshly prepared;

5. **Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) with the registered substance;**

6. **Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance;**

7. **Long-term toxicity testing on fish (Annex VIII, Section 9.1.3., column 2; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance.**

8. **Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: CO2 evolution test, OECD TG 301B) or**

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: MITI test (I), OECD TG 301C) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Closed bottle test, OECD TG 301D) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Manometric respirometry test, OECD TG 301F) or

   Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Ready biodegradability – CO2 in sealed vessels (headspace test), OECD TG 310) with the registered substance

You have to submit the requested information in an updated registration dossier by **6 October 2020**. You also have to update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3.

Appeal

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

Authorised[1] by **Claudio Carlon**, Head of Unit, Hazard Assessment C3

[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 1: Reasons

SUBSTANCE IDENTITY

1. **High-pressure liquid chromatogram, gas chromatogram (Annex VI, Section 2.3.6.)**

In accordance with Article 10(a)(ii) of the REACH Regulation, the technical dossier must contain information on the identity of the substance as specified in Annex VI, Section 2 to the REACH Regulation. In accordance with Annex VI, Section 2 the information provided has to be sufficient to enable the identification of the registered substance.

According to Annex VI, section 2.3.6 of the REACH Regulation, the registration needs to contain a chromatogram (Gas Chromatogram, or High Pressure Liquid Chromatogram). In addition, according to the Guidance for identification and naming of substances under REACH and CLP (May 2017, Version 2.1), a chromatogram needs to be provided, where appropriate depending on the type of substance considered, to confirm the composition of the registered substance. For example, an appropriate chromatogram will confirm the existence of impurities, additives and the constituents of a reaction mixture. For HPLC chromatograms the following information should be indicated on the chromatogram itself or in annexes (ECB, 2004; ECB, 2005):

- The identity of the substance;
- Column properties, such as diameter, packing, length;
- Temperature, also temperature range if used;
- Composition of the mobile phase ,also range if used;
- Concentration range of the substance;
- Visualisation method, e.g. UV-VIS;
- Results (indicate the main peaks important for substance identification).

In section 1.4 of your technical dossier you provided some chromatographic information (file named "[redacted]" containing a HPLC/UV method, and file named "[redacted]" containg a GC/MS method) used for the quantification of the impurity "2-chloro-4-nitroaniline", but no chromatogram was provided. Moreover, no analytical method and analytical data were provided for the quantification of the main constituent 1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol.

Hence, your dossier does not provide adequate information to verify the composition of the registered substance and therefore its identity.

Therefore, you need to provide adequate chromatographic data to support the composition of your substance as reported in section 1.2. The data must include the method description together with the chromatogram and corresponding peak table with the identification of the peaks, peak areas and area %. The identification and values of each peak (main constituent and impurities) provided in the chromatographic report need to be consistent with the information reported in section 1.2 so that the composition of the substance can be verified. The requested data should be attached in IUCLID section 1.4.

You did not provide any specific comment on this request in your comments on the draft decision.

TOXICOLOGICAL AND ECOTOXICOLOGICAL INFORMATION

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 10 to 100 tonnes10 to 100 tonnes per year must contain, as a minimum, the information specified in Annexes VII to VIII Annexes VII to VIII to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Your registration dossier contains for multiple endpoints adaptation arguments in the form of a grouping and read-across approach according to Annex XI, Section 1.5. of the REACH Regulation. ECHA has assessed first the scientific and regulatory validity of your grouping and read-across approach in general before the individual endpoints (sections 3, 5 and 6).

Grouping of substances and read-across approach

You have sought to adapt information requirements by applying a read-across approach in accordance with Annex XI, Section 1.5, for the endpoints:

- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.),
- Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.),
- Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2),
- Ready biodegradability (Annex VII, Section 9.2.1.1).

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). ECHA considers that the generation of information by such alternative means should offer equivalence to prescribed tests or test methods.

Based on the above, a read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological or ecotoxicological property is reliable and should be based on recognition of the structural similarities and differences between the source and registered substances[2]. This hypothesis explains why the differences in the chemical structures should not influence the toxicological and ecotoxicological properties or should do so in a regular pattern. The read-across approach must be justified scientifically and documented thoroughly, also taking into account the differences in the chemical structures. There may be several lines of supporting evidence used to justify the read-across hypothesis, with the aim of strengthening the case.

Due to the different nature of each endpoint and consequent difference in scientific considerations (e.g. key parameters, biological targets), a read-across must be specific to the endpoint or property under consideration. Key physicochemical properties may determine the fate of a compound, its partitioning into a specific phase or compartment and largely influence the availability of compounds to organisms, e.g. in bioaccumulation and toxicity tests. Similarly, biotic and abiotic degradation may alter the fate and bioavailability of compounds as well as be themselves hazardous, bioaccumulative and/or persistent. Thus, physicochemical and degradation properties influence the human health and environmental properties of a substance and should be considered in read-across assessments. However, the information on physicochemical and degradation properties is only a part of the read-

[2] Please see for further information ECHA *Guidance on information requirements and chemical safety assessment* (version 1, May 2008), Chapter R.6: QSARs and grouping of chemicals.

across hypothesis, and it is necessary to provide additional justification which is specific to the endpoint or property under consideration.

The ECHA Read-across assessment framework foresees that there are two options which may form the basis of the read-across hypothesis[3]- (1) (Bio)transformation to common compound(s)- the read-across hypothesis is that different substances give rise to (the same) common compounds to which the organism is exposed and (2) Different compounds have the same type of effect(s)- the read-across hypothesis is that the organism is exposed to different compounds which have similar (eco)toxicological and fate properties as a result of structural similarity (and not as a result of exposure to common compounds).

Finally, Annex XI, Section 1.5. lists several additional requirements, which deal with the quality of the studies which are to be read-across.

You consider to achieve compliance with the REACH information requirements for the registered substance 1-[(2-chloro-4-nitrophenyl)azo]-2-naphthol (EC number: 220-562-2, referred as C.I. PIGMENT RED 4 or PR4) using data of structurally similar substances 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC number: 219-372-2, referred as C.I. PIGMENT RED 3 or PR3) and 1-[(2,4-dinitrophenyl)azo]-2-naphthol (EC number: 222-429-4, referred as C.I. PIGMENT ORANGE 5 or P05) (hereafter the 'source substances').

You have provided a read-across documentation in section 1.2. of the Chemical Safety Report (CSR).

You use the following arguments to support the prediction of properties of the registered substance from data for source substances within the group: the substances show similarity in their structure, their physico-chemical properties and environmental fate as well as similar toxicological and eco-toxicological properties. You specify that "*the category are synthesized in the same manner by* [redacted]. *Therefore the category members share a similar impurity profile*". You also mention the following: "*Lacking bioavailability is probably the reason for the absence of any relevant mammalian toxicity. 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 properties in any study (OECD473, 476, 482) except in Ames assays (OECD 471). Furthermore, Monoazo Red Pigments do not exert toxic effects to aquatic, terrestrial and sediment organisms as well as bacteria. […]*" and you conclude that "*structural similarities with very similar physical-chemical properties, environmental fate, ecotoxicity and mammalian toxicity enable the treatment of these Monoazo Red Pigments as a category and fulfilment of data requirements by read across from one category members to all other category members is justified*". Furthermore, you provide a data matrix summarizing available data on the three members of proposed category (section 1.3. of the CSR). As an integral part of this prediction, you propose that the source and registered substance(s) have similar properties for the above-mentioned information requirements. ECHA considers that this information is your read-across hypothesis.

ECHA's evaluation and conclusion

Your proposed adaptation argument is that the similarity in chemical structure and in some of the physico-chemical, ecotoxicological and toxicological properties between the source and registered substance is a sufficient basis for predicting the properties of the registered

[3] Please see ECHA's Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

substance for other endpoints. Structural similarity is a prerequisite for applying the grouping and read-across approach. However similarity in chemical structure and similarity of some of the physico-chemical, ecotoxicological and toxicological properties does not necessarily lead to predictable or similar human health and environmental properties in other endpoints. Your justification based on structural similarity, similar physico-chemical, ecotoxicological and toxicological properties has not established why the prediction is reliable for the human health and environmental endpoints for which the read across is claimed.

Additionally, ECHA has taken into account all of your arguments together. ECHA firstly notes that you have not provided a reasoning as to why these arguments add to one another to provide sufficient basis for read-across. Secondly, the defects of each individual argument are not mitigated by the other arguments you have provided, and so ECHA considers that the arguments when taken all together do not provide a reliable basis for predicting the properties of the registered substance.

Therefore, ECHA considers that this grouping and read-across approach does not provide a reliable basis whereby the human health effects and environmental effects of the registered substance may be predicted from data for reference substance(s) within the group. Hence, this approach does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. of the REACH Regulation. ECHA notes that there are specific considerations for the individual endpoints which also result in a failure to meet the requirement of Annex XI, Section 1.5., and these are set out under the endpoint concerned.

As described above, further elements are needed to establish a reliable prediction for a toxicological or ecotoxicological property, based on recognition of the structural similarities and differences between the source and registered substances. This could be achieved (if it is possible) by a well-founded hypothesis of (bio)transformation to a common compound(s), or that the registered and source substance(s) have the same type of effect(s), together with sufficient supporting information to allow a prediction of human health and environmental properties.

Toxicological endpoints

2. Short-term repeated dose toxicity (28 day), one species (Annex VIII, Section 8.6.1.)

A "short-term repeated dose toxicity study (28 days)" is a standard information requirement as laid down in Annex VIII, Section 8.6.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided a study record for a repeated dose toxicity study in rat via the oral route (claimed equivalent to OECD TG 408, not GLP). However, this study does not provide the information required by Annex VIII, Section 8.6.1., because it is not reliable and therefore it fails to meet the requirements of Annex XI, 1.1.2. Accordingly, this study cannot be used to adapt the information requirement for this endpoint according to Annex VIII, Section 8.6.1., column 2.

More specifically, ECHA notes that while you identify this study as key study with a reliability score of 2, you indicate the following: *"Unknown purity, old and not very well described method, Not GLP"*. In addition, ECHA notes the following:

- The purity of the test substance is unknown;
- The study does not include testing of at least three dose levels, appropriate measurements (weighing at least once a week, food and water consumption) and observations of relevant parameters (ophtalmological examination, haematology, clinical biochemistry and urinalysis);
- The information reported on gross necropsy and histopathology (on kidney and spleen) is not considered to provide enough details.

Therefore, your adaptation of the information requirement is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

When there is no information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407), nor for the screening study for reproductive/developmental toxicity (OECD TG 421 or TG 422 as explained below under point 3.), 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.[4]

ECHA has evaluated the most appropriate route of administration for the study. ECHA considers that both inhalation and oral routes may be adequate for this substance (given physicochemical properties and uses). The inhalation route is appropriate as the substance is a solid with a low vapour pressure (0.2 10^{-9} Pa at 25°C) defined by a QSAR model and uses may warrant exposure by inhalation of workers during manufacturing (PROC 4, 8b) and formulation (PROC 5, 8b, 9, 14, 15, 24) because transfer and mixing of substance are described. The substance also has uses at industrial sites (PROC 5, 6, 7, 8a, 10, 13, 14, 21, 24), by professional workers (SU 7, 17, 19) and finally it has consumer uses (PC 9a, PC 18, PC 32).

However, in your waiver for the acute inhalation study, you claimed that "*The test substance has very low vapor pressure and high melting point, so the potential for the generation of inhalable forms is low, also the use of this substance will not result in aerosols, particles or droplets of an inhalable size, so exposure to humans via the inhalatory route will be unlikely to occur, and no acute inhalation test was performed*". The oral route is appropriate as (i) mortality occurred in an acute toxicity study with the registered substance (3/10 test animals were found dead at 10 g/kg bw) and (ii) systemic effects were observed after oral exposure (diet) in the abovementionned repeated dose toxicity study.

Based on the information provided in the technical dossier and in the chemical safety report (very low solubility, no irritation, mainly used as pigment included into matrice, particle size mainly in the respirable fraction), and after balancing all the arguments presented above ECHA considers that the oral route - which is the preferred one as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 6.0, July 2017) Chapter R.7a, section R.7.5.4.3 - is the most appropriate route of administration.

[4] 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)

More specifically, even though the substance is reported to be present as a dust with a significant proportion of particles of respirable size (D50 < 2.3 µm), it is used when incorporated in a matrix, and so concern for inhalation exposure is minimal. In addition, the available oral studies indicate a concern for systemic toxicity which requires further information on repeated dose toxicity by the oral route.

According to the test methods OECD TG 422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with rats.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

You did not provide any specific comment on this request in your comments on the draft decision.

3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

"Screening for reproductive/developmental toxicity" (test method OECD TG 421 or 422) is a standard information requirement as laid down in Annex VIII, Section 8.7.1. of the REACH Regulation if there is no evidence from available information on structurally related substances, from (Q)SAR estimates or from *in vitro* methods that the substance may be a developmental toxicant. No such evidence is presented in the dossier. Therefore, adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a screening for reproductive/developmental toxicity (OECD TG 421, GLP compliant) with the source substance 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC number: 219-372-2, i.e. PR3).

However, as explained above in Appendix 1, section "Grouping of substances and read-across approach" of this decision, your adaptation of the information requirement is rejected.

Therefore, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

When there is not information available neither for the 28-day repeated dose toxicity endpoint (EU B.7, OECD TG 407) (as explained above under point 2.), 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.[5]

[5] 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)

According to the test methods OECD TG 422, the test is designed for use with rats. On the basis of this default assumption ECHA considers testing should be performed with rats.

As explained in Section 2 above, based on the information provided in the technical dossier and in the chemical safety report ECHA considers that the oral route is the most appropriate route of administration.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (test method: OECD TG 422) in rats by the oral route.

You did not provide any specific comment on this request in your comments on the draft decision.

4. In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) OR Transgenic rodent somatic and germ cell gene mutation assays (Annex VIII, Section 8.4., column 2)

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "Appropriate *in vivo* mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII".

The technical dossier contains two *in vitro* studies for *in vitro* gene mutation in bacteria (Ames tests) performed according to OECD TG 471 with the registered substance that show a positive and an ambiguous result. More specifically, the positive result was observed in the TA 98 strain after metabolic activation, and you concluded *that "PR4 induced gene mutations by frameshifts in the genome of the strain TA 98 in the presence of metabolic activation. Therefore, C.I Pigment Red 4 is considered to be mutagenic in* this Salmonella typhimurium *and* Escherichia coli *reverse mutation assay".* The positive result indicates that the substance is inducing gene mutations under the conditions of the test. ECHA notes that no test was submitted following the "Prival protocol" which is better suited to assess the monoazodyes (see OECD TG 471, 1997, paragraph 10).

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the registered substance.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, October 2017) Chapter R.7a, Section R.7.7.6.3, 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. Hence, ECHA considers that the TGR and the comet assay are suitable tests to follow up the concern on gene mutation for the substance subject to the decision.

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 substance is usually administered orally. 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 ºC) 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.

Moreover, ECHA notes that 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. Hence, in order to limit additional animal testing male germ cells shall be collected at the same time as the other tissues (liver, glandular stomach and duodenum) , and stored up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, in accordance with Annex IX/X, Section 8.4., column 2, 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.

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. 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision:

Transgenic rodent somatic and germ cell gene mutation assays (test method: EU B.58/ OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and 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. The test material used should be freshly prepared.

OR

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

Notes for your consideration

You are reminded that according to Annex IX/X, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including

toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered".

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

You did not provide any specific comment on this request in your comments on the draft decision.

Aquatic toxicity endpoints

ECHA notes that, according to the information provided in your technical dossier, the registered substance shall be regarded as poorly water soluble (the reported water solubility in as resported under section 4.8 of your IUCLID dossier is 3.3 µg/L). ECHA considers that substances that are poorly soluble in water require longer time to be significantly taken up by the test organisms and so steady state conditions are likely not to be reached within the duration of a short-term toxicity test. For this reason, short-term tests may not give a true measure of toxicity for such substances and toxicity may actually not even occur at the water solubility limit of the substance if the test duration is too short.

Accordingly, the information requirements as specified un Annex VII, Section 9.1.1, column 2 and Annex VIII, Section 9.1.3., column 2 apply. Therefore, long-term toxicity needs to be investigated already at the tonnage band currently applicable for the substance subject to the present decision.

5. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

"Growth inhibition study aquatic plants" is a standard information requirement as laid down in Annex VII, Section 9.1.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

Column 2 of Annex VII, Section 9.1.2 specifies that the study does not need to be conducted if there are mitigating factors indicating that aquatic toxicity is unlikely to occur for instance if the substance is highly insoluble in water or the substance is unlikely to cross biological membranes.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for an algae growth inhibition test (OECD TG 201, GLP compliant) with the source substance 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC number: 219-372-2, i.e. PR3). The results showed no toxicity, with a 72-hour NOErC ≥ 6 µg/L (measured concentration).

However, as explained above in Appendix 1, section "Grouping of substances and read-across approach" of this decision, your adaptation of the information requirement is rejected.

Accordingly, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Therefore there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) Algae growth inhibition test (test method EU C.3. / OECD TG 201) is the preferred test to cover the standard information requirement of Annex VII, Section 9.1.2.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Algae growth inhibition test, EU C.3./OECD TG 201).

In your comments on the draft decision you agreed to conduct the requested study.

6. Long-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1., column 2)

As explained above, for poorly water soluble substance, "Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex VII, Section 9.1.1., column 2 of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for long-term toxicity test on aquatic invertebrates test (OECD TG 211, GLP compliant) with the source substance 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EC number: 219-372-2, i.e. PR3). The results showed no toxicity, with a 21-day NOEC ≥ 35 µg/L (measured concentration).

However, as explained above in Appendix 1, section "Grouping of substances and read-across approach" of this decision, your adaptation of the information requirement is rejected.

Accordingly, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Therefore there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) *Daphnia magna* reproduction test (test method EU C.20. / OECD TG 211) is the preferred test to cover the standard information requirement for a long-term toxicity test on aquatic invertebrates.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *Daphnia magna* reproduction test (test method: EU C.20./OECD TG 211).

In your comments on the draft decision you agreed to conduct the requested study.

7. Long-term toxicity testing on fish (Annex VIII, Section 9.1.3., column 2)

For poorly water soluble substances, "Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex VIII, Section 9.1.3., column 2 of the REACH Regulation. Adequate information on Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.), or Fish, short-term toxicity test on embryo and sac-fry stages (Annex IX, 9.1.6.2.), or Fish, juvenile growth test (Annex IX, 9.1.6.3.) needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to according to Annex IX, Section 9.1.6., column 2. You provided the following statement: "*Waiving "column 2" in Annex IX of REGULATION (EC) No 1907/2006 (long-term toxicity test on daphnia available - CSA does not indicate need for further investigations)*".

However, ECHA considers that your adaptation does not meet the specific rules for adaptation of Annex VIII, Section 9.1.3., column 2. Indeed, as explained previously in section 4 and 5, ECHA notes that there is currently no reliable aquatic toxicity data in your registration dossier. In addition, you did not provide any justification that there are mitigating factors indicating that aquatic toxicity is unlikely to occur.

ECHA also points out that your statement that "*CSA does not indicate need for further investigations*" is not considered a valid justification to adapt the information requirement for this endpoint. First, ECHA notes that none of the studies reported in section 6.1. of your technical dossier were conducted on the registered substance and that, as explained above in Appendix 1, section "Grouping of substances and read-across approach" of this decision, your adaptation of the information requirement is rejected. In addition, as already explained above under section "Aquatic toxicity endpoints", short-term data would not be considered as reliable source of information to characterize the toxicity of the registered substance to aquatic organisms as it is poorly water soluble. Accordingly, it cannot be concluded wether fish, invertebrates or aquatic plants might be substantially more sensitive. Finally, your CSA dossier does not include a reliable exposure assessment and risk charcaterization supporting that toxicity is unlikely to occur.

Therefore, your adaptation of the information requirement cannot be accepted.

In your comments on the draft decision, you indicate that you intend to evaluate the need to conduct a long-term toxicity study on fish once the results of the growth inhibition study on aquatic plants and the long-term toxicity study on aquatic invertebrates will be available. You consider that, if no effects are observed up to the water solubility limit in these two toxicity tests, it is unlikely that toxic effects will be identified in a long-term toxicity study on fish. You further claim that the registered substance will not affect the aquatic or terrestrial environment as the substance is either used in applications where no release to these compartments is foreseen or is included in matrices with low expected release.

As already explained above, the technical dossier does not contain any information to support a (or lack of) sensitivity difference among aquatic organisms. Furthermore, based on the uses reported in the technical dossier, ECHA considers that such uses are reported for which exposure of the aquatic environment cannot be excluded (e.g. Environmental

Release Category (ERC) 8c/8f). Hence, you did not demonstrate that there are mitigating factors indicating that aquatic toxicity is unlikely to occur.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.
According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) fish early-life stage (FELS) toxicity test (test method OECD TG 210), fish short-term toxicity test on embryo and sac-fry stages (test method EU C.15. / OECD TG 212) and fish juvenile growth test (test method EU C.14. / OECD TG 215) can be performed to cover the standard information requirement of Annex IX, Section 9.1.6.

However, the FELS toxicity test according to OECD TG 210 is more sensitive than the fish, short-term toxicity test on embryo and sac-fry stages (test method EU C.15 / OECD TG 212), or the fish, juvenile growth test (test method EU C.14. / OECD TG 215), as it covers several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (see ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), *Chapter R7b, Section R.7.8.4.1.*

Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are expected to cause effects over a longer exposure period, or which require a longer exposure period of time to reach steady state (ECHA *Guidance Chapter R7b,* version 4.0, June 2017).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

Notes for your consideration

As already explained, ECHA notes that no reliable short-term studies available on aquatic invertebrates or on fish for the registered substance can be generated as the substance is poorly water soluble. Therefore the Integrated testing strategy (ITS) outlined in ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R7b (Section R.7.8.5 including Figure R.7.8-4), is not applicable in this case and the long-term studies on both invertebrates and fish are requested to be conducted. As the registered substance has a reported low water solubility, long-term studies are indicated.

8. Ready biodegradability (Annex VII, Section 9.2.1.1.)

"Ready biodegradability" is a standard information requirement as laid down in Annex VII, section 9.2.1.1. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex XI, Section 1.5. of the REACH Regulation by providing a study record for a Ready Biodegradability: Modified MITI Test (I) (OECD TG 301 C) with the analogue substance 1-[(4-methyl-2-nitrophenyl)diazenyl]-2-naphthol (EC No 219-372-2; ie PR3)

However, as explained above in Appendix 1, section "Grouping of substances and read-across approach" of this decision, your adaptation of the information requirement is rejected.

Therefore, your adaptation of the information requirement cannot be accepted.

Furthermore, the read-across study submitted does not provide the information required by Annex VII, Section 9.2.1.1., because, contrary to Article 3(28) of the REACH Regulation, the documentation of the study is insufficient and does not allow an independent assessment of its adequacy, results and use for hazard assessment. In particular, as the full study report is available mainly in Japanese and not fully available in EU language, it is not possible to evaluate the study report.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

Regarding the test method, depending on the substance profile, you may conclude on ready biodegradability, by applying the most appropriate and suitable test guideline among those listed in the ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) and in the paragraph below. The test guidelines include the description of their applicability domain.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to perform one of the following tests with the registered substance subject to the present decision:

Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: CO2 evolution test, OECD TG 301B)
or
Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: MITI test (I), OECD TG 301C)
or
Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Closed bottle test, OECD TG 301D)
or
Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Manometric respirometry test, OECD TG 301F)
or
Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: Ready biodegradability – CO2 in sealed vessels (headspace test), OECD TG 310) with the registered substance

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 2 August 2017.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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

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.

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

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-63 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

2. 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 your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.