

Helsinki, 9 April 2018

Addressee: [REDACTED]

Decision number: CCH-D-2114394043-52-01/F

Substance name: 3-hydroxy-N-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide

EC number: 229-440-3

CAS number: 6535-46-2

Registration number: [REDACTED]

Submission number: [REDACTED]

Submission date: 05.11.2015

Registered tonnage band: 1000+T

**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. Sub-chronic toxicity study (90-day), inhalation route (Annex IX, Section 8.6.2.; test method: OECD TG 413) in rats modified to include analysis of bronchoalveolar lavage (BAL) analysis (as further specified in Appendix 1 (1)) with the registered substance;**
- 2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;**
- 3. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rat or rabbit), oral route with the registered substance;**
- 4. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
  - Ten weeks pre-mating exposure duration for the parental (P0) generation;**
  - Dose level setting shall aim to induce some toxicity at the highest dose level;**
  - Cohort 1A (Reproductive toxicity);**
  - Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;<sup>1</sup>**

<sup>1</sup> No testing for an extended one-generation reproductive toxicity study may be started or performed at this moment: You may start performing the extended one-generation reproductive toxicity study only after [exact date - 15 months from the date of the decision] unless ECHA has communicated to you to do otherwise.

- 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 IX, Section 9.1.5.; test method: Daphnia magna reproduction test, EU C.20./OECD TG 211) with the registered substance;**
- 7. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance;**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI to the REACH Regulation. To ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective annex, and adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **18 October 2021** except for the information requested under point 1 for a sub-chronic toxicity study (90-day) which shall be submitted in an updated registration dossier by **16 April 2019**. You may only commence the extended one-generation reproductive toxicity study as requested under point 4 after **16 July 2019**, unless an indication to the contrary is communicated to you by ECHA before that date. You shall also 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<sup>2</sup> by **Claudio Carlon**, Head of Unit, Evaluation **E2**

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<sup>2</sup> 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**

As a preliminary remark we would like to address your comments on ECHA's draft decision whereby you argued that your substance is not a nanomaterial according to the Commission recommendation of 18 October 2011 on the definition of a nanomaterial. In the light of all this, you indicated that *"any requirements just based on the assumption the registered material could be a nanomaterial are seen as not proportionate and cannot be accepted"*.

However, following your comments, ECHA has modified the draft decision so that the present decision does not refer to or imply that your substance is fulfilling the conditions set out in the Commission recommendation on the definition of a nanomaterial. Accordingly, none of the study requested is justified by the fact that your substance would be a nanomaterial. These requests are only justified by the fact that your dossier contains data gaps in relation to these information requirements.

In this context, ECHA understands that your comment on the draft decision may result from the indication, particularly in Appendix 3 of the decision, that the test material record must document as a minimum the constituent concentration values and any other parameter that is relevant (for instance the size or the shape of the particles). More specifically, some sections of Annex I point out, where appropriate, to specific OECD guidance documents that ECHA believe appropriate to consider when performing the requested studies due to the characteristics of your substance. Accordingly, these sections refer to OECD GD No. 23 (2000), OECD GD No. 36 (2012) and OECD expert report No 40 (2014). The OECD GD No. 23 (2000) is not specific to nanomaterials but it addresses more generally substances that are difficult to test. The OECD GD 36 (2012) and OECD report No 40 (2014) provide advice for manufactured nanomaterials, however ECHA considers that this guidance applies more generally to small particle that are poorly soluble. Providing the reference to these OECD documents is therefore a reasonable recommendation to make in order to ensure that any new studies conducted represent the best available advice also for poorly soluble and small or indeed very small particle substance.

ECHA stresses that, in any case, appropriate identification of a test material is a general requirement set out in OECD test guidelines, including the test guidelines referred to in the present decision. Given that a registered substance may vary in its parameters (e.g. purity, composition, size, etc.) and that the nature of the test sample may impact the results of a toxicological study, it is essential to consider and document such parameters when selecting the test material. This applies to all materials, regardless of size. In addition, the OECD TG 413 specifically requires reporting of the particle size distribution of the test sample. This is relevant for all particles, regardless of whether they are nanomaterials or not. Moreover, according to the OECD TG 413, additional characterization information that are relevant to particulates, in particular nanomaterials, includes shape, surface area/specific surface area, surface chemistry, composition including coating and surface modifications, surface charge, particle solubility, and aggregation/agglomeration state.

### **1. Sub-chronic toxicity study (90-day), inhalation route (Annex IX, Section 8.6.2.)**

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

A "sub-chronic toxicity study (90 day)" is a standard information requirement as laid down in Annex IX, Section 8.6.2. 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 not provided any study record of a sub-chronic toxicity study (90 day) in the dossier that would meet the information requirement of Annex IX, Section 8.6.2. In the technical dossier you have provided a study record for a "repeated dose 28-day oral toxicity study" (test method: OECD TG 407). However, this study does not provide the information required by Annex IX, Section 8.6.2., because exposure duration is less than 90 days and the number of animals per dose group is significantly lower. Therefore, the sensitivity of a 28-day study is much lower than that of a 90-day study. Additionally, the 28-day oral study does not cover all the parameters normally foreseen to be investigated in a sub-chronic toxicity study (90 days) via inhalation route.

In addition to the 28-day study, you have sought to adapt this information requirement according to Annex IX, Section 8.6.2., column 2 and Annex XI, Section 1.2. You provided the following justification for the adaptation:

*"Consistent with Section 8.6.2 of REACH Annex IX and in accordance with Section 1.2 of REACH Annex XI, there is sufficient weight of evidence from several independent sources of information leading to the conclusion that Pigment Red 112 does not cause toxicity after repeated oral administration, including the 90-day period, and thus does not have to be classified, because*

- *Pigment Red 112 is a chemically unreactive substance,*
- *Pigment Red 112 can be considered insoluble because it has an extremely low solubility in water and n-octanol,*
- *due to its extremely low solubility, it is unlikely that Pigment Red 112 becomes systemically bioavailable after oral, dermal or inhalation exposure,*
- *Pigment Red 112 caused no systemic toxic effects in a 28-day oral gavage study in rats (NOAEL 1000 mg/kg/day) and there was no evidence of absorption of the substance,*
- *Pigment Red 112 does not have to be classified as skin sensitizing (referring to the pigment as such notwithstanding possible effects of impurities) or as skin or eye irritating, indicating that its chemical inertness and extremely low solubility in water and n-octanol largely prevent interaction with living cells and tissues,*
- *in the unlikely event of workplace exposure to aerosolized pigment in respirable form, the substance is considered likely to behave like an inert dust; from the use and exposure pattern long-term inhalation exposure is not considered relevant for the general population. It can therefore be concluded with sufficient certainty that Pigment Red 112 will not cause toxicity after repeated oral administration, including the 90-day period, and that testing in a 90-day study is not scientifically necessary".*

ECHA notes that although you have sought to adapt this information requirement using a weight of evidence approach, the elements used to justify your weight of evidence adaptation are nearly identical to the criteria of the column 2 specific rules of adaptation of Annex IX, Section 8.6.2, fourth indent, which states that the sub-chronic toxicity study (90 days) does not need to be conducted if:

*"the substance is unreactive, insoluble and **not inhalable** and there is no evidence of absorption and no evidence of toxicity in a 28-day 'limit test', particularly if such a pattern is coupled with limited human exposure".*

ECHA considers that although the adaptation is marked as a weight of evidence adaptation, it in fact relies on the same elements in the specific rule for adaptation noted above. According to section 1.2 of Annex XI of REACH, the weight of evidence adaptation enables a registrant to fulfil an information requirement by relying on "*several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to support this notion*". ECHA notes that the "several sources of information" you invoke in the dossier are simply the different conditions set out in the column 2 adaptation of Annex IX, Section 8.6.2, fourth indent. Therefore, the use of the weight of evidence label cannot be used to set a different threshold for accepting the adaptation set out in column 2 of Annex IX, Section 8.6.2, as the justification provided in the dossier does not bring in any additional elements for consideration.

In this light, ECHA notes that your adaptation does not meet the conditions of the specific rules for adaptation of Annex IX, Section 8.7.2., column 2 for the following reasons:

- The substance itself is inhalable, as evidenced by the particle size distribution of the substance, where the key study shows that the D50 for the substance is 4.5 µm. Other (non-key studies) included in your dossier also show the D50 of the substance ranging from 0.0903 µm (number based distribution of dispersed particles), to 6.49 µm (size distribution of the aggregates and agglomerates of the undispersed powder). Therefore, the substance does not meet the criterion of being not inhalable as required by the specific rules of adaptation of Annex IX, Section 8.6.2, fourth indent.
- Your justification states that the substance is considered unreactive. However, no supporting evidence has been provided for this claim. This applies equally to the use of this argument in the context of the specific rule for adaptation.
- Your justification considers that the substance has low solubility in octanol and water. Although the water solubility of your substance is low (9.8 µg/L), the substance shows some solubility in octanol (3.31 mg/L). This solubility in octanol, and the octanol-water partition coefficient of 2.5 for this substance indicates the potential for absorption of the substance. Therefore, the available information for the substance contradicts your justification, as well as the criteria in the specific rule for adaptation for this endpoint.
- In addition, although the substance has low solubility in water, and some solubility in octanol, this information is in itself not sufficient to demonstrate lack of absorption or dissolution of the substance in the lung environment following inhalation exposure or the gastrointestinal tract following oral exposure.

Therefore, your adaptation according to Annex IX, Section 8.7.2., column 2 of the information requirement is rejected.

In addition to evaluating your adaptation against the specific rules for adaptation of Annex IX, Section 8.6.2, fourth indent, your justification also refers to the lack of toxicity in the available 28-day study and irritation studies. This argument goes beyond the conditions set out in the adaptation in column 2 of Annex, Section 8.6.2, fourth indent. You may have considered that this further argument would requalify an argumentation under column 2 of Annex, Section 8.6.2, fourth indent, into a weight of evidence argumentation. ECHA notes,

however, that the conditions set out in column 2 of Annex, Section 8.6.2, fourth indent, are not met. The toxicity or absorption in the 28-day study is not sufficient to compensate the failure to meet these conditions in the form of a weight of evidence.

ECHA has for the sake of completeness also evaluated your adaptation with respect to weight of evidence provision as well. This is because you have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence and your weight of evidence argument appears based on the specific rules of adaptation (as noted above).

You have provided the following sources of individual information:

- Key study: short term repeated dose toxicity study (28 days), in rats, via the oral route (OECD TG 407; GLP) with the registered substance, NOTOX, 2008 (study report [REDACTED]), rel. 1.

You have concluded (as quoted above) that the substance does not cause toxicity after repeated dose exposure in a 90-day repeated dose oral toxicity study.

ECHA has evaluated your weight of evidence information according to REACH Annex XI, Section 1.2., and has assessed whether you have provided "*sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that the substance has or has not a particular dangerous property*" with respect to the information requirement of Annex IX, Section 8.6.2. for a sub-chronic toxicity study, considering both oral and inhalation routes.

ECHA has further evaluated the information according to ECHA Guidance R.4.4. by considering whether the criteria given in that guidance i.e. relevance, reliability and adequacy for the purpose apply to the information you have provided. In its assessment, ECHA has considered the investigations, the number of animals used in the studies provided and the consistency in the effects presented across the lines of information. ECHA came to a view on whether the set of information presented addresses the properties of the substance by covering, as a minimum, the most relevant elements investigated in a sub-chronic toxicity study (OECD TG 408 or OECD TG 413).

ECHA notes that a number of elements of the 90-day repeated dose toxicity study are not sufficiently addressed by the available 28-day repeated dose toxicity study. These include:

- *The number of animals: the available 28-day repeated dose toxicity study used 5 animals per sex per dose, whereas a 90-day repeated dose toxicity study requires the use of 10 animals per sex per dose. Therefore, the statistical power regarding to most elements is low reducing the confidence,*
- *The duration of the study is 28 days, compared to 90 days required for this endpoint,*
- *Considerations of the most appropriate route of administration and related parameters.*

Therefore, ECHA concludes that the provided study does not provide adequate and reliable coverage of the key investigation foreseen in the corresponding test method, i.e. an exposure duration that is comparable or longer than the corresponding method, route of administration, and statistical power. ECHA notes that none of the other arguments included in your justification (lack of solubility, reactivity, etc.) address these missing elements.

Hence, ECHA considers that the individual sources of information you provided, taken together with your justification for the adaptation, do not allow to assume/conclude that the substance does not have a particular dangerous property with respect to the information requirement for Annex IX, Section 8.6.2

Therefore, ECHA concludes that the general rules for adaptation laid down in Annex XI, Section 1.2. of the REACH Regulation are not met and your adaptation of the information requirement is rejected.

Based on the 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.

ECHA has evaluated the most appropriate route of administration for the study. The information provided in the technical dossier and the chemical safety report on properties of the registered substance and its uses indicate that human exposure to the registered substance by the inhalation route is likely. More specifically, the substance is considered to behave as an inert dust with a significant proportion (>1% on weight basis) of particles of inhalable size (MMAD < 50 µm). Furthermore, the substance is respirable, of low water solubility and consequently there is a potential for accumulation of the substance in the lungs.

Hence, the test shall be performed by the inhalation route using the test method OECD TG 413.

According to the test method OECD TG 413 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat. The registered substance is a dust that is highly insoluble in water. The results of the particle size distribution provided in the registration dossier indicate that > 50% (by mass) of particles are less than 10 µm.

In that respect, ECHA does note that the size of particles may impact the performance of certain studies (e.g. if a substance is composed of small particles rather than large particles, this may impact the selection of the route of exposure for repeated dose toxicity studies).

ECHA notes that all the particle size distribution studies referred to in your comments show that the substance is composed of small particles. This information should be taken into account in designing the requested studies in order to ensure that the results are appropriate for this substance.

Since the provided data on the substance solubility in water and particle size distribution indicate that the lower respiratory tract (i.e., the alveoli) might be the primary site of deposition and retention of the registered substance subject to the present decision and no information is yet available to characterize the risk, ECHA is requesting that bronchioalveolar lavage (BAL) is being performed in the test. BAL fluid shall be analyzed for total and differential cell count, protein content and lactate dehydrogenase. You should consider other parameters taking into account potential effects of the substance in the lung. You should further consider that the preferred mode of exposure is nose-only and that particulate materials should be subjected to mechanical processes. Particle sizing should be performed for all aerosols and for vapours that may condense to form aerosols. To allow for exposure of all relevant regions of the respiratory tract, aerosols with mass median aerodynamic diameters (MMAD) ranging from 1 to 3 µm with a geometric standard

deviation (og) in the range of 1.5 to 3.0 are recommended. Further guidance on mechanical processes to decrease the particle size is provided in Guidance Document on Acute Inhalation Toxicity Testing, Environmental Health and Safety Monograph Series on Testing and Assessment No. 39, ENV/JM/MONO(2009)28, OECD TG, Paris. This Guidance document indicates that especially for particles deemed to be innocuous and biologically "inert," emphasis should be given to generating particle size distributions amenable to preferentially depositing in the lower respiratory tract. For such materials it is recommended that MMADs ranging from 0.1 to 2 µm be used in repeated exposure studies to maximize lung exposure in rodent studies.

In your comments on the draft decision, you stated that prolonged exposure of rats to an insoluble dust via inhalation route would produce lung overload effects. Therefore you proposed an alternative approach including 1) to modify the route of administration for the sub-chronic toxicity study from inhalation to oral to show the absence of systemic effects, to show the possible metabolism of the test material by gut bacteria under the anaerobic, reductive conditions of the large intestine, which could lead to exposure to reactive metabolites of the azo-compound that would not occur in an inhalation study and 2) an additional short-term inhalation study including BAL assessment with extended recovery to evaluate potential specific hazards while avoiding the risk of artificial lung-overload effects. ECHA reminds that the revised TG 413 – (adopted 9 October 2017) accommodates the testing of solid aerosols.

The revised TG 413 recommends measurements of lung burden when a range-finding and/or main study or other relevant information suggests that a solid aerosol is poorly soluble and likely to be retained in the lung; hence, avoiding the risk of artificial lung-overload effects. In your comment on the draft decision, you argued that the oral route would include the possible metabolism of the test material by gut bacteria under the anaerobic, reductive conditions of the large intestine, which could lead to exposure to reactive metabolites of the azo-compound that would not occur in an inhalation study. ECHA however notes that the potential exposure to possible reactive metabolites of the azo-compound by gut bacteria will be assessed in the requested reproductive studies performed via the oral route.

In your comment on the draft decision, you also stated that human exposure is mainly confined to the process of filling and emptying containers under controlled conditions and with appropriate protective equipment. ECHA however notes that no exposure assessment has been provided in the dossier to verify this statement.

Regarding your comment on providing an additional short-term inhalation study including BAL assessment to the 90-day oral study, ECHA reminds that the duration of a short term inhalation study is not comparable to a sub-chronic 90-day study and it does not give the same information as a long-term study does (e.g. histopathology and clinical chemistry parameters).

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: Sub-chronic inhalation toxicity: 90-day study (test method: OECD TG 413) in rats. The test shall be performed using nose-only exposure and shall include bronchoalveolar lavage (BAL) analysis.

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



Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

A "pre-natal developmental toxicity study" (test method EU B.31./OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. 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.

The technical dossier does not contain information on a pre-natal developmental toxicity study with the registered substance.

You have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence. Hence, ECHA has evaluated your adaptation with respect to this provision. You have not provided any studies for this endpoint. Instead, you have provided the following justification for the adaptation:

*"In accordance with Section 1.2 of REACH Annex XI, there is sufficient weight of evidence from several independent sources of information leading to the conclusion that the substances of this category do not cause developmental toxicity and thus does not have to be classified, because*

- the substances of this category did not cause lethal effects after administration of a single oral or dermal dose of  $\geq 2000$  mg/kg in rats,*
- the substances of this category do not have to be classified as eye or skin irritating,*
- the substances of this category caused no relevant systemic toxic effects in several subacute oral studies in rats (NOAEL  $\geq 1000$  mg/kg/day),*
- the substances of this category caused no relevant systemic toxic effects in parental animals and offspring in a Reproduction/Developmental Toxicity Screening test and a Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening in rats (NOAEL  $\geq 1000$  mg/kg/day),*
- the absence of effects in the above-mentioned toxicity endpoint tests indicates that the substances of this category do not interact with living cells/tissues, and*
- it is unlikely that the substances of this category become systemically bioavailable due to their extremely low solubility in water and low solubility in n-octanol,*

*It can therefore be concluded with sufficient certainty that the substances of this category will not cause developmental toxicity and that testing performed on one or two species is not scientifically necessary".*

ECHA notes that your proposed adaptation is qualified as a weight of evidence. The weight of evidence adaptation requires several independent sources of information leading to the assumption/conclusion on the properties of the substance. However, ECHA notes that the "several sources of information" you invoke in the dossier are simply the different conditions set out in the column 2 adaptation of Annex IX/X, Section 8.7.2, third indent. Therefore, the use of the weight of evidence qualification cannot be used to set a different threshold for accepting the adaptation set out in column 2 of Annex IX/X, Section 8.7.2, third indent, as the justification provided in the dossier does not bring in any additional elements for consideration.

More specifically, Annex IX/X, Section 8.7.2, column 2, 3<sup>rd</sup> indent, states that the study does not need to be conducted if:

*“the substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) and there is no or no significant human exposure”.*

However, you have not provided any *“toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air)”*. Therefore, while there is no evidence of toxicity seen in the available tests, ECHA considers that your arguments regarding low solubility and expected low absorption/biological availability do not meet the criteria established in the specific rule for adaptation noted above.

Moreover, the same considerations as for the sub-chronic repeated dose toxicity apply here regarding these arguments for low solubility and expected low absorption/biological availability.

In addition, ECHA notes that the anaerobic environment of the lower gastrointestinal tract of mammals is well suited for azo-reduction. This is of particular relevance when the substance is administered via the oral route, as is normally the case for pre-natal developmental toxicity studies. Although parent compounds may not be absorbed, or may have limited absorption, a potential exposure to any metabolites that can be formed in the lower intestine cannot be excluded. There is no evidence from the available repeated dose study in the dossier whether metabolites were formed or not and the assumption of no absorption was not demonstrated.

Therefore, your proposed adaptation does not meet the specific criteria for adaptation in column 2 of Annex IX/X, Section 8.7.

In addition to evaluating your adaptation against the specific rules for adaptation of Annex IX, Section 8.7.2., third indent, ECHA has for the sake of completeness also evaluated your adaptation with respect to weight of evidence provision as well. This is because you have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence and your weight of evidence argument appears based on the specific rules of adaptation (as noted above).

Accordingly, ECHA has assessed whether you have provided *“sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that the substance has or has not a particular dangerous property”* with respect to the information requirement of Annex IX, Section 8.7.2. for a pre-natal developmental toxicity study.

ECHA has further evaluated the information according to ECHA Guidance R.4.4. by considering whether the criteria given in that guidance i.e. relevance, reliability and adequacy for the purpose apply to the information you have provided. In its assessment, ECHA has considered the information on the key investigations, number of animals used in the studies provided and the consistency in the effects presented across the lines of information. ECHA came to a view on whether the set of information presented addresses

the properties of the substance by covering, as a minimum, the most relevant elements investigated in a pre-natal developmental toxicity study (EU B.31/OECD TG 414).

ECHA notes that you have not provided any studies under this endpoint and your adaptation does not provide adequate and reliable coverage of any of the key information of a pre-natal developmental toxicity study.

ECHA further notes that while you state that "*the substances of this category caused no relevant systemic toxic effects in parental animals and offspring in a Reproduction/Developmental Toxicity Screening test and a Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening in rats (NOAEL  $\geq$  1000 mg/kg/day)*", no such studies were provided in the dossier and also no category approach was elaborated.

Therefore, in the absence of any substantiating documentation, this mere statement cannot be considered as a relevant source of information as part of a weight of evidence adaptation.

Finally, none of the other arguments in your weight of evidence adaptation addresses the key elements of a pre-natal developmental toxicity study.

In your comments on the draft decision, you stated that based on the unreactive, almost insoluble, and non-toxic character of the test material no significant internal exposure of the reproductive organs and effects on the development of the offspring are not expected. You also proposed an oral kinetic study and stated that reproductive toxicity studies should only be considered if bioavailability has been proven. Furthermore, you referred to an OECD TG 421 study on C.I. Pigment Red 170 showing no adverse effects.

However, your comments refers firstly to arguments initially submitted in your registration dossier in relation to the subchronic toxicity study. These arguments are already addressed in section 1 of the present appendix. In summary, ECHA points out that the substance does not meet the criterion of being not inhalable; no supporting evidence has been provided to substantiate the claim that the substance is considered unreactive; the available information in the dossier on solubility in octanol contradicts the allegation that the substance is poorly soluble; finally, the information on poor solubility is in itself not sufficient to demonstrate lack of absorption or dissolution of the substance in the lung environment following inhalation exposure or the gastrointestinal tract following oral exposure. Secondly, ECHA stresses that it has not been proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air). Thirdly, the 28-day endpoint study record in IUCLID does not contain data on reproductive organs. Neither are the data of the OECD TG 421 study on C.I. Pigment Red 170 available in the dossier.

Based on the above, ECHA considers that your comments on the draft decision are not such as to alter the conclusion that the weight of evidence adaptation proposed is not valid. ECHA concludes that the evidence you provided to adapt the information requirement for an pre-natal developmental toxicity study based on Annex XI, Section 1.2. is not sufficient to assume/conclude that the substance has not dangerous/hazardous properties with regard to this endpoint. 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.

According to the test method EU B.31./OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits as a first species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA Guidance on information requirements and chemical safety assessment (version 5.0, December 2016) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid/dust, ECHA concludes that testing should be performed by the oral route.

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: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a first species (rat or rabbit) by the oral route.

### **3. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species**

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Pre-natal developmental toxicity studies (test method EU B.31./OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier does not contain information on a pre-natal developmental toxicity study with the registered substance.

You have sought to adapt this information requirement, with the same adaptation used for the pre-natal developmental toxicity study in a first species. As explained above, 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.

According to the test method EU B.31./OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default consideration, ECHA considers testing should be performed with rabbits or rats as a second species, depending on the species tested in the first pre-natal developmental toxicity study.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA Guidance on information requirements and chemical safety assessment

(version 5.0, December 2016) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid/dust, ECHA concludes that testing should be performed by the oral route.

In your comments on the draft decision you invoked animal welfare and the need to perform a study in rabbits only if there are equivocal indications of a developmental hazard in a previous rat study. You also claim that significant internal exposure of the reproductive organs and effects on the development of the offspring are not expected due to the unreactive, almost insoluble, and non-toxic character of the test material.

Concerning your comments, ECHA stresses that, under Section 8.7.2 of Annex X of the REACH Regulation, registrants are required to perform a developmental toxicity study on a second species, unless the adaptations set out in Column 2 of Section 8.7 of Annex X and Annex XI justify that such a test is not necessary. However, the reasons invoked in your comments do not provide a valid justification for such adaptations. More specifically, these arguments are already addressed in section 1 and 2 of the present appendix. In summary, ECHA points out that the substance does not meet the criterion of being not inhalable; no supporting evidence has been provided to support the claim that the substance is considered unreactive; the available information in the dossier on solubility in octanol contradicts the allegation that the substance is poorly soluble; finally, the information on poor solubility is in itself not sufficient to demonstrate lack of absorption or dissolution of the substance in the lung environment following inhalation exposure or the gastrointestinal tract following oral exposure.

Based on the above, ECHA considers that your comments on the draft decision are not such as to alter the conclusion that the adaptation proposed is not valid.

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: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species (rat or rabbit) by the oral route.

Notes for your consideration

You are reminded that before performing a pre-natal developmental toxicity study in a second species you must consider the specific adaptation possibilities of Annex X, Section 8.7., column 2 and general adaptation possibilities of Annex XI. If the results of the test in the first species with other available information enable such adaptation, testing in the second species should be omitted and the registration dossier should be updated containing the corresponding adaptation statement.

#### **4. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)**

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information

requirement as laid down in column 1 of 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3.

Further detailed guidance on study design and triggers is provided in the ECHA *Guidance on information requirements and chemical safety assessment R.7a*, chapter R.7.6 (version 5.0, December 2016).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

*a) The information provided*

You have sought to adapt this information requirement according to Annex XI, Section 1.2. weight of evidence. You provided the following justification for the adaptation:

*"In accordance with Section 1.2 of REACH Annex XI, there is sufficient weight of evidence from several independent sources of information leading to the conclusion that Pigment Red 112 does not cause toxicity to reproduction and thus does not have to be classified, because*

- Pigment Red 112 is a chemically unreactive substance,*
- Pigment Red 112 can be considered insoluble because it has an extremely low solubility in water and n-octanol,*
- due to its extremely low solubility, it is unlikely that Pigment Red 112 becomes systemically bioavailable after oral, dermal or inhalation exposure,*
- Pigment Red 112 caused no systemic toxic effects in a 28-day oral gavage study in rats (NOAEL 1000 mg/kg/day) and there was no evidence of absorption of the substance,*
- Pigment Red 112 does not have to be classified as skin sensitizing (referring to the pigment as such, notwithstanding possible effects of impurities) or as skin or eye irritating, indicating that its chemical inertness and extremely low solubility in water and n-octanol largely prevent interaction with living cells and tissues,*

*It can therefore be concluded with sufficient certainty that Pigment Red 112 will not cause toxicity to reproduction and that testing is not scientifically necessary".*

ECHA notes that your proposed adaptation is qualified as a weight of evidence. The weight of evidence adaptation requires several independent sources of information leading to the assumption/conclusion on the properties of the substance. However, ECHA notes that the "several sources of information" you invoke in the dossier are simply the different conditions set out in the column 2 adaptation of Annex X, Section 8.7., third indent. Therefore, the use of the weight of evidence qualification cannot be used to set a different threshold for accepting the adaptation set out in column 2 of Annex X, Section 8.7., third indent, as the justification provided in the dossier does not bring in any additional elements for consideration.

Annex X, Section 8.7., column 2, 3<sup>rd</sup> indent, which states that the study does not need to be conducted if:

*"the substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of*

*metabolites of the substance in urine, bile or exhaled air) and there is no or no significant human exposure".*

However, you have not provided any *"toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air)." Therefore, while there is no evidence of toxicity seen in the available tests, ECHA considers that your arguments regarding low solubility and expected low absorption/biological availability do not meet the criteria established in the specific rule for adaptation noted above. In addition, the same considerations as for the sub-chronic repeated dose toxicity apply here regarding these arguments for low solubility and expected low absorption/biological availability.*

In addition, ECHA notes that the anaerobic environment of the lower gastrointestinal tract of mammals is well suited for azo-reduction. This is of particular relevance when the substance is administered via the oral route, as is normally the case for Extended one-generation reproductive toxicity studies. Although parent compounds may not be absorbed, or may have limited absorption, a potential exposure to any metabolites that can be formed in the lower intestine cannot be excluded. There is no evidence from the available repeated dose study in the dossier whether metabolites were formed or not and the assumption of no absorption was not demonstrated.

Therefore, your proposed adaptation does not meet the specific criteria for adaptation in column 2 of Annex X, Section 8.7.

In addition to evaluating your adaptation against the specific rules for adaptation of Annex X, Section 8.7., third indent, ECHA has for the sake of completeness also evaluated your adaptation with respect to weight of evidence provision as well. This is because you have sought to adapt this information requirement according to Annex XI, Section 1.2., weight of evidence and your weight of evidence argument appears based on the specific rules of adaptation (as noted above).

ECHA has evaluated your weight of evidence information according to REACH Annex XI, Section 1.2., and assessed whether you have provided *"sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that the substance has or has not a particular dangerous property"* with respect to the information requirement of Annex X, Section 8.7.3. for the registered substance (see 'specification of the study design').

ECHA has further evaluated the information according to ECHA Guidance R.4.4. by considering whether the criteria given in that guidance i.e. relevance, reliability and adequacy for the purpose apply to the information you have provided. In its assessment, ECHA has considered the information on key investigations, number of animals used in the studies provided and the consistency in the effects presented across the lines of information. ECHA came to a view on whether the set of information presented addresses the properties of the substance by covering, as a minimum, the most relevant elements investigated in an extended one-generation reproductive toxicity study (EU B.56./OECD TG 443).

More specifically, ECHA considers that this study provides relevant information on two aspects, namely on sexual function and fertility in parental P0 and F1 generation (further

referred to as "sexual function and fertility") and on developmental toxicity observable peri- and postnatally in F1 generation (further referred to as "post-natal developmental toxicity").

The following elements are most relevant for the registered substance to address "sexual function and fertility": 10 weeks pre-mating exposure, male and female reproductive performance (such as gonadal function, mating behaviour, conception, development of the conceptus and parturition), sperm parameters, oestrus cycles, and histopathological examinations of reproductive organs of the P0 generation and sperm parameters, oestrus cycle and histopathological examinations of reproductive organs of the F1 generation in early adulthood.

The following elements are most relevant for the registered substance to address "post-natal developmental toxicity": peri- and post-natal investigations of the F1 generation up to post natal day 90 of age (such as growth, survival/mortality, certain external malformations, investigations related to hormonal modes of action like anogenital distance, nipple retention, thyroid hormone measurements, and sexual maturation).

You have provided no experimental data with respect to any of the relevant aspects listed above and the arguments provided in your adaptation do not address any of these key elements.

In your comments on the draft decision you also invoked animal welfare and the need to perform a reproductive toxicity study only if there are unequivocal indications of a reproductive hazard by proven bioavailability. In addition, you claim that significant internal exposure of the reproductive organs and effects on the development of the offspring are not expected due to the unreactive, almost insoluble, and non-toxic character of the test material. You also proposed an oral kinetic study and you referred to an OECD TG 421 study on C.I. Pigment Red 170 showing no adverse effects. Moreover, you claimed that the 28-day study provides no indications for the formation and/or absorption of possible cleavage products such as o-toluidine or 2,4,5-trichloro-aniline since typically associated toxic effects are completely missing.

However, your comments refer firstly to arguments initially submitted in your registration dossier in relation to the subchronic toxicity study. These arguments are already addressed in section 1, 2 and 3 of the present appendix. In summary, ECHA points out that no supporting evidence has been provided to support the claim that the substance is considered unreactive; the available information in the dossier on solubility in octanol contradicts the allegation that the substance is poorly soluble; finally, the information on poor solubility is in itself not sufficient to demonstrate lack of absorption or dissolution of the substance in the gastrointestinal tract following oral exposure. Secondly, ECHA stresses that it has not been proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure (e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air). Thirdly, the 28-day endpoint study record in IUCLID does not contain data on reproductive organs. Neither are the data of the OECD TG 421 study on C.I. Pigment Red 170 available in the dossier.

Based on the above, ECHA considers that your comments on the draft decision are not such as to alter the conclusion that the weight of evidence adaptation proposed is not valid. Therefore, in light of the lack of information on the key elements normally investigated in this study and the insufficient evidence for your claims in your adaptation justification, it



is not possible to conclude that the substance has or has not a particular dangerous/hazardous property, in this case reproductive toxicity as specified by Annex XI; Section 1.2. Consequently, 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. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

*b) The specifications for the study design*

*Information from studies to be conducted before the extended one-generation reproductive toxicity study*

The sub-chronic toxicity study shall be conducted before the extended one-generation reproductive toxicity study and the results from that study shall be used, among to other relevant information, to decide on the study design of the extended one-generation reproductive toxicity study following ECHA *Guidance on information requirements and chemical safety assessment R.7a*, chapter R.7.6 (version 5.0, December 2016).

The sub-chronic toxicity study may provide information on effects that is relevant for triggers (e.g. weight changes and histopathological observations of organs as indication(s) of one or more modes of action related to endocrine disruption which may meet the toxicity-trigger for extension of Cohort 1B or as evidence of specific mechanism/modes of action and/or neurotoxicity and/or immunotoxicity which may meet the particular concern criteria for developmental neurotoxicity and/or developmental immunotoxicity cohorts).

*Premating exposure duration and dose-level setting*

To ensure that the study design adequately addresses the fertility endpoint, the duration of the pre-mating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA *Guidance*, the starting point for deciding on the length of pre-mating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks pre-mating exposure duration is required because there is no substance specific information in the dossier supporting shorter pre-mating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment R.7a*, chapter R.7.6 (version 5.0, December 2016).

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a conducted range-finding study (or range finding studies) are reported with

the main study. This will support the justifications of the dose level selections and interpretation of the results.

#### *Species and route selection*

According to the test method EU B.56./ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 5.0, December 2016) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a solid/dust, ECHA concludes that testing should be performed by the oral route.

#### c) Outcome

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: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks pre-mating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;

Currently, the extension of Cohort 1B and the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) are not requested. However, the sub-chronic toxicity study (90-day) requested in this decision (request 1) and/or any other relevant information may trigger changes in the study design. Therefore, the sub-chronic toxicity study (90-day) is to be conducted first and the study results submitted to ECHA in a dossier update by **16 April 2019**. If, on the basis of this update and/or other relevant information, a need for changes to the study design is identified, ECHA will inform you by **16 July 2019** (i.e. within three months after expiry of the 12-month deadline to provide the sub-chronic toxicity study (90-day)) of its intention to initiate a new decision making procedure under Articles 41, 50 and 51 of the REACH Regulation to address the design of the extended one-generation reproductive toxicity study. If you do not receive a communication from ECHA by **16 July 2019**, the request of the present decision for the extended one-generation reproductive toxicity study remains effective and you may commence the conduct of the study and the results will need to be submitted by the deadline given in this decision **18 October 2021**.

#### *Notes for your consideration*

When submitting the study results of the sub-chronic toxicity study (90-day) you are invited to also include in the registration update your considerations whether changes in the study design are needed (see also ECHA *Guidance on information requirements and chemical*

*safety assessment R.7a, chapter R.7.6 (version 5.0, December 2016)).*

Furthermore, after having commenced the extended one-generation reproduction toxicity study in accordance with the ECHA decision, you may also expand this study to address a concern identified during the conduct of it and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the changes in the study design must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/ triggers must be documented.

#### **5. Growth inhibition study on aquatic algae and cyanobacteria (Annex VII, Section 9.1.2.)**

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

“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.

In the technical dossier you have provided a study record for a key study based on an OECD TG 201 (GLP, 2010), from [REDACTED] performed according to the OECD Guidance document (GD) 23 for aquatic difficult to test substances . You reported effect value of EC50 > 1 mg/L (nominal concentration, saturated solution). You provided as summary the following information:

*“The toxicity of Pigment Red 112 (batch number 2468) to the unicellular freshwater green algae *Desmodesmus subspicatus* was determined according to the principles of OECD 201 at [REDACTED] from January 08 to 14, 2010 with the definitive exposure phase from January 11 to 14, 2010. The aim of the study was to assess the effects on growth rate and yield over a period of 72 h.*

*The study was conducted under static conditions with an initial cell density of approximately  $2 - 5 \times 10^3$  cells/mL. Based on a preliminary range finding test, a limit test with a saturated solution\* was carried out. The saturated solution was prepared out of the dispersion of 1 mg/L. Six replicates were tested for the limit concentration and control. Environmental conditions were determined to be within the acceptable limits. All effect values are given based on the nominal concentration of the test item.”*

However, this study does not provide the information required by Annex VII, Section 9.1.2., because the applied preparation method described in OECD GD 23 is not applicable for the registered substance and leads to uncertainty on the exposure concentration.

ECHA notes that the registered substance is composed of particles that are poorly soluble and small or very small. ECHA further notes that the OECD GD 23<sup>3</sup> specifies which type of

<sup>3</sup> OECD (2000) Guidance Document on aquatic toxicity testing of difficult substances and mixtures. OECD Series on testing and assessment No. 23, ENV/JM/MONO(2000)6. <http://www.oecd-ilibrary.org/docserver/download/9750231e.pdf?expires=1503564027&id=id&accname=guest&checksum=12155F51EAB543672958ACC1A4D3DB45>

dispersions are covered in the guidance. In that respect, the OECD GD 23 is not a suitable guidance for preparation of the test dispersion for poorly water soluble particles. Indeed, the application of the dispersion preparation as per OECD GD 23, may lead to substantial decrease in the concentration of the test substance in the media and lead to unacceptable level of uncertainty in the exposure concentration. The results were provided only as nominal concentration (saturated solution). Therefore, ECHA concludes that the level of exposure to the test organisms cannot be verified and therefore the results are not acceptable.

In your comments on the draft decision, you considered that the provided study on growth inhibition on aquatic plants (OECD TG 201) is fully valid and requesting an additional OECD 201 study is not justified. You also stated that the experimental approach based on the best scientific judgment followed the OECD Guidance 23 as follows: substance concentration of 1 mg/L was stirred for 48 hours in the aqueous test medium. You considered this solution stable. You also stated that due to low water solubility (9.8 µg/L) of the substance they could not establish any analytical methods with an acceptable effort. You concluded that as the substance is assumed to be stable (persistent and has a low adsorption potential), solubility limit can be used as the exposure concentration.

Moreover, ECHA acknowledges your statement that the method was selected using best scientific judgement at the time of testing (2010). However, ECHA considers that you did not provide adequate justification to support the selection of the method in relation to the substance properties and behaviour.

*At best, in your comments you also noted that "Generally, organic pigments are powdered materials, which are almost insoluble in water and in most organic solvents. The pigments, normally having a broad particle size distribution, are strongly agglomerated or are aggregated. Additionally they are only poorly dispersible at least in aqueous media."*

ECHA notes that in the robust study summary you provided information that after mixing the 1 mg/L dispersion for 48 hour to gain saturated solution, the dispersion was filtrated with 0.45 µm RC filter. However, no analytical information on the concentration of the test substance in the start or end of the test was provided. ECHA considers that due to the substance properties described in the comments and technical dossier, poor water solubility and tendency to agglomerate or aggregate, the filtration step may have removed majority the test substance from the test media. Therefore, ECHA considers that the uncertainty of the exposure concentration remains and the filtration step is not recommended when aquatic toxicity of particulate substances is assessed. Hence, the OECD GD 23 is not considered applicable in these circumstances. Instead, the sample needs to be prepared without filtration, as prescribed in OECD Guidance 36<sup>4</sup> and report Series on the Safety of Manufactured Nanomaterials No. 40<sup>5</sup>. Indeed, these documents provide advice on methods applicable for particles and poorly water soluble substance which are small particles or very small particles.

<sup>4</sup> OECD (2012) Guidance on Sample Preparation and dosimetry for the safety testing of manufactured nanomaterials. Series on the Safety of Manufactured Nanomaterials, No. 36, ENV/JM/MONO(2012)40.

[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2012\)40&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)40&doclanguage=en)

<sup>5</sup> OECD (2014) Expert Meeting Report - Ecotoxicology and environmental fate of manufactured nanomaterials: test guidelines.

Series on the Safety of Manufactured Nanomaterials, No. 40, ENV/JM/MONO(2014)1.

[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2014\)1&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)1&doclanguage=en)

Finally, in your comments on the draft decision, you also stated that "*since the substance is not an engineered nanomaterial, there is no need to follow a specific guidance for these materials*". While taking note of your comment, ECHA considers the reference to OECD GD 36 and OECD No 40 to be justified as the registered substance is composed of poorly soluble small particles.

Furthermore, the stimulation of the growth in the test suspension was not explained in the technical dossier. According to the OECD TG 201 a growth stimulation (negative inhibition) can result from either hormesis ("toxic stimulation") or from addition of stimulating growth factors with the test material to the minimal medium used. Low dose stimulation can usually be ignored in EC50 calculations unless it is extreme. However, this effect should be taken into account in assessment of the study results.

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 3.0, February 2016) 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)

## **6. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)**

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. 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 key study based on an OECD TG 211, performed using the OECD GD 23 (GLP, 2010). The test suspension was prepared by mixing 1 mg/L of the test substance in water (20 rpm, 48 hours) followed by filtration (0.45 µm). It was reported that saturated solution was used as a test solution. As a result you reported NOELR of 1 mg/L nominal loading rate (saturated solution).

However, this study does not provide the information required by Annex IX, Section 9.1.5., because the applied preparation method is not applicable for the registered substance and leads to uncertainty on the exposure concentration. ECHA notes that the registered substance is composed of poorly soluble particles that are small or, indeed, very small. As described under section 5 of appendix 1 ECHA further considers that due to the substance properties described in the comments and technical dossier, poor water solubility and

tendency to agglomerate or aggregate, the filtration step may have removed majority of the test substance from the test media. Therefore, ECHA considers that the uncertainty of the exposure concentration remains and the filtration step is not recommended when aquatic toxicity of particulate substances is assessed. Hence, the OECD GD 23 is not considered applicable in these circumstances. Instead, the sample needs to be prepared without filtration, as prescribed in OECD Guidance 36<sup>6</sup> and report Series on the Safety of Manufactured Nanomaterials No. 40<sup>7</sup>. Indeed, these documents provide advice on methods applicable for particles and poorly water soluble substance which are small particles or, indeed, very small particles. The application of the dispersion preparation as per OECD GD 23, may lead to substantial decrease in the concentration of the test substance in the media and lead to unacceptable level of uncertainty in the exposure concentration. The results were provided only as nominal concentration (saturated solution). Therefore, ECHA concludes that the level of exposure to the test organisms cannot be verified and therefore the results are not acceptable.

In your comments to the draft decision, you consider that the provided study on long-term toxicity to aquatic invertebrates (OECD TG 211) is fully valid and requesting an additional OECD 211 study is not justified.

As indicated above under request 5, ECHA considers that, due to the selected aquatic toxicity test design and the lack of monitoring and interpretation of the results, there is still uncertainty concerning the exposure of the organisms to the tested substance and therefore the reliability of the test results and interpretation

Consequently, for the reasons explained above, there is an information gap and hence it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 3.0, February 2016) *Daphnia magna* reproduction test (test method EU C.20. / OECD TG 211) is the preferred test to cover the standard information requirement of Annex IX, Section 9.1.5.

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: Long-term toxicity testing on aquatic invertebrates (Annex IX, 9.1.5.; test method: *Daphnia magna* reproduction test, EU C.20/OECD 211).

## **7. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)**

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

"Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. Adequate information on Fish, early-life

<sup>6</sup> OECD (2012) Guidance on Sample Preparation and dosimetry for the safety testing of manufactured nanomaterials. Series on the Safety of Manufactured Nanomaterials, No. 36, ENV/JM/MONO(2012)40.

[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono\(2012\)40&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)40&doclanguage=en)

<sup>7</sup> OECD (2014) Expert Meeting Report - Ecotoxicology and environmental fate of manufactured nanomaterials: test guidelines.

Series on the Safety of Manufactured Nanomaterials, No. 40, ENV/JM/MONO(2014)1.

[http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO\(2014\)1&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)1&doclanguage=en)

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 the information requirements according to Annex IX, Sections 9.1.6 column 2. You provided the following justification for the adaptation: "*Waiving according to "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 notes that your adaptation does not meet the specific rules for adaptation of Annex IX, Section 9.1.6., column 2 because due to the substance properties and lack of information on the short term toxicity to aquatic organisms at this stage the chemical safety assessment (CSA) is not complete. Therefore, ECHA considers that the CSA cannot be used to justify that there is no need for further information on toxicity to fish.

ECHA notes that there are no short-term studies available on aquatic invertebrates or on fish for the registered substance. Therefore the Integrated testing strategy (ITS) outlined in ECHA Guidance on information requirements and chemical safety assessment (version 3.0, February 2016), 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. ECHA further notes that the registered substance has low water solubility (9.8 µg/L) and information on short term aquatic toxicity would not be suitable to define the PNEC for the aquatic compartment. According to column 2 of Annex VIII, Section 9.1.3 long term aquatic test with fish shall be considered when the substance is poorly water soluble.

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

In your comments to the draft decision, you disagree on the need for information on long-term effects on fish. You were of the opinion that you have provided adequate information regarding the aquatic toxicity with OECD TG 201 and OECD TG 211 results. You considered that these long-term studies are valid and can be used in the assessment and therefore that the study on vertebrate animals is not justified. In addition, you claim that due to low water solubility, the requested study OECD TG 210, would not provide any added value for the assessment and CSA.

However, information on "Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. Currently, there is no information available in the technical dossier on toxicity to fish. ECHA notes that for the derivation of the PNEC<sub>aquatic</sub> data on three trophic levels, on aquatic invertebrates, fish and aquatic plants, is required (ECHA Guidance on information requirements and chemical safety assessment, v.4.0, June 2017, Chapter R7b, Section R.7.8.5.3).

ECHA agrees with you that due to the poor water solubility of the registered substance short-term tests would not provide adequate information. Furthermore, Annex VIII 9.1.3. and Annex VII 9.1.1. of the REACH Regulation explicitly recommend that long-term aquatic toxicity tests be considered if the substance is poorly water soluble. Therefore long-term data on all three trophic levels is needed for the derivation of PNEC<sub>aquatic</sub> and to perform the chemical safety assessment. In addition, ECHA considers that due to the low water solubility, the short-term data cannot serve as a compelling evidence to predict relative differences (or lack of) in species sensitivity.

Due to the reasons outlined above, it is not possible to define the order of sensitivities of the three species as would be required to apply the aquatic ITS (ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), Section R.7.8.5.3.).

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 3.0, February 2016) 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) are the preferred tests 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 3.0, February 2016), *Chapter R7b, Figure R.7.8-4*).

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 3.0, February 2016).

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).

*Note for consideration for aquatic testing (request 4. to 6.)*

ECHA notes that the registered substance is composed of poorly soluble particles that are small or, indeed, very small. Due to this characteristic, you are required to apply the following OECD documents: Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials ENV/JM/MONO(2012)40 and Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines ENV/JM/MONO(2014)1 when preparing the test material or performing the requested test.

Once results of the test on long-term toxicity to fish are available, you shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation.



## **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 17 August 2016.

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 amended the request(s).

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

As no amendments were proposed, ECHA took the decision according to Article 51(3) 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 request(s) in this decision, or to fulfil otherwise the information requirement(s) 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 in relation to Annex VII-XI data requirements, the sample of the substance used for the new test(s) 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 substance as actually manufactured or imported by each registrant.

Finally there must be adequate information on substance identity for the sample tested and the substance registered to enable the relevance of the tests to be assessed. For each study record reported, adequate information on the test material used to generate the data needs to be documented in the test material record linked to the EndPoint Study Record. The test material record will document as a minimum the constituent concentration values and any other parameter that is relevant (for instance the size or the shape of the particles). The registrants' rationale for the choice of each representative test material will be given in sufficient detail so that its relevance and representativeness for the registered substance can be independently verified. Technical instructions are available in the Manual "How to prepare registration and PPORD dossiers" on the ECHA website [https://echa.europa.eu/documents/10162/22308542/manual\\_regis\\_and\\_ppord\\_en.pdf](https://echa.europa.eu/documents/10162/22308542/manual_regis_and_ppord_en.pdf).