

Helsinki, 18 February 2020

Addressees

Registrants of [REDACTED] listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of this decision

07/05/2013

Registered substance subject to this decision, hereafter 'the Substance'

Substance name: N,N'-phenylene-1,4-bis[4-[(2,5-dichlorophenyl)azo]-3-hydroxynaphthalene-2-carboxamide]

EC number: 223-460-6

CAS number: 3905-19-9

Decision number: [Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/D)]**DECISION ON A COMPLIANCE CHECK**Based on Article 41 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of **23 November 2022**.**A. Requirements applicable to all the Registrants subject to Annex VII of REACH**

1. Same in vivo genotoxicity study as request C.1 (triggered by Annex VII, Section 8.4., column 2)

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

1. Same in vivo genotoxicity study as request C.1 (triggered by Annex VIII, Section 8.4., column 2)
2. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487), with the Substance.
3. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method OECD 421/422) in rats, oral route, with the Substance.

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

1. In vivo genotoxicity study to be selected according to the following scenarios:
 - a. If the test results of request B.2 are negative:

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

OR

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

b. If the test results of request B.2 are positive:

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

2. Sub-chronic toxicity study (90-day), inhalation route (Annex IX, Section 8.6.2.; test method OECD TG 413) in rats , with the Substance. The study must include measurements of lung burden and bronchoalveolar lavage fluid (BALF) analysis as described in the current version (25 June 2018) of the OECD TG 413.
3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method OECD TG 414) in a first species (rat or rabbit), oral route, with the Substance;

Conditions to comply with the requests

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

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

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

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

The same information is required from registrants at several appendices of this decision. The reasons for (triggering of) the requested information are provided in the corresponding appendices while the selection and design of the requested studies is examined in Appendices A and C.

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

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

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

Appeal

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

Authorised¹ under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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

Appendix on general considerations

(i) Assessment of the Grouping of substances and read-across approach, in light of the requirements of Annex XI, Section 1.5.

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

- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

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

Grouping of substances and read-across approach

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

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

A. Scope of the grouping

i. Description of the grouping

In your registration dossier you refer to a category of 'disazocondensation red pigments'. You have provided a read-across/category justification documentation in sections of the CSR (toxicokinetic, discrete endopoints).

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

Abbreviation/Name	CAS number
1) PB23 /Pigment Brown 23	35869-64-8
2) PB41 /Pigment Brown 41	68516-75-6
3) PR144 /Pigment Red 144	5280-78-4
4) PR166 /Pigment Red 166	3905-19-9
5) PR214 /Pigment Red 214	40618-31-3
6) PR220 /Pigment Red 220	68259-05-2
7) PR221 /Pigment Red 221	71566-54-6
8) PR242 /Pigment Red 242	52238-92-3
9) PR262 /Pigment Red 262	79665-24-0

As reasons for grouping the substances you argue that they are not bioavailable and thus of no toxicological relevance due to their low solubility in different media and large molecular size.

You define the structural basis for the grouping as "disazocondensation red pigments". ECHA understands that this is the applicability domain of the grouping and will assess your predictions on this basis.

In your comments to the draft decision you clarify that your intention is not to define a category but to use "*read-across approach*" to adapt individual information requirements. ECHA acknowledges your clarifications. On that basis, ECHA understands that you submitted an analogue approach. The basis for predictions, which apply to an analogue approach as part of a read-across adaptation, is discussed further below under B. *Predictions for properties*; these additional explanations do not change the outcome of ECHA's assessment.

B. Predictions for properties

You have provided the following reasoning for the prediction of toxicological properties: The "*category hypothesis is fundamentally based on the low bioavailability. None of the pigments are sufficiently soluble either in water or in octanol for systemic uptake or metabolism.*" [...] You argue this based on the molecular weight (██████████) and modelled rigidity of the structures, low solubility (<0.1 mg/L) and "*no increased solubility or degradation in stomach acid is possible.*" You further consider a potential metabolism of the analogue substances, which would lead to metabolites of higher toxicity.

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substance.

You intend to predict the properties for the analogue substances from information obtained from valid studies² with the following analogue substances:

- I. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.) similar or according to OECD TG 471:
 - a. PR144 (CAS 5280-78-4), 2006
 - b. PR166 (CAS 3905-19-9), 2006
- II. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.) similar or according to OECD TG 473
 - a. PR242 (CAS 52238-92-3), 1992
- III. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.) similar or according to OECD TG 476.
 - a. PR166 (CAS 3905-19-9), 1989
- IV. Repeated dose toxicity (Annex VIII-IX, Section 8.6)
 - a. PR166 (CAS 3905-19-9) according to OECD TG 407, 2009
 - b. PR220 (CAS 68259-05-2), according to OECD TG 422, 2012
- V. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1) and Prenatal developmental toxicity (Annex IX, Section 8.7.2)
 - a. PR220 (CAS 68259-05-2), according to OECD TG 422, 2012

ECHA notes the following shortcomings with regards to predictions of toxicological properties.

1. Missing supporting information

² You have provided further *in vivo* genotoxicity studies, which ECHA considers invalid or inappropriate for the relevant endpoints, for reasons explained in the the endpoint section, below.

Annex XI, Section 1.5 of the REACH Regulation states that "*adequate and reliable documentation of the applied method shall be provided*". Within this documentation "*it is important to provide supporting information to strengthen the rationale for the read-across*"³. The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

"*Adequate and reliable documentation*" must include

- i. supporting information on the absence of bioavailability and
- ii. bridging studies to compare such properties of the analogue substances.

As indicated above, your read-across hypothesis is based on the assumption that the structurally similar target and source substances cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the target and source substance is necessary to confirm that both substance cause the same type of effects. Such information can be obtained, for example, from bridging studies, and from studies demonstrating absence of bioavailability.

Supporting information on the absence of bioavailability

In your read-across hypothesis, you state that the analogue substances have a molecular weight between [REDACTED] as well as low solubility in water and organic solvents, which results in a very low bioavailability and thus no or low toxicity. Furthermore, you have submitted short-term toxicity studies on two of nine analogue substances, PR166 and PR220, which demonstrate no effect levels at the limit dose of 1000 mg/kg bw/d. This data set reported in the technical dossier does not include relevant, reliable and adequate information for the target and the source substances to support your read-across hypothesis.

In your comments to the draft decision:

- you consider the "*existing information is sufficient.*"
- you refer to "*structurally similar pigments*" and state "*any uptake would have resulted in either detection of the colored substance in tissues (non-metabolized) or in severe amine-related toxicity (destruction of the chromophore by metabolism).*" You also point to pigment red 53:1 (EC 225-935-3) and pigment red 48:2 (EC 230-303-5) as examples of pigments that have coordination bonds with a cation that will disintegrate in the acidic environment of the stomach and known to cause systemic toxicity. You did not provide the related data (e.g. robust study summaries of the relevant studies) in your documentation.
- you refer to the presence or absence of systemic or reproductive toxicity in numerous available repeated dose toxicity and reproductive toxicity studies on structurally variable types of pigments outside the scope of the category which are available in the ECHA database. You did not explain the relevance of the indicated supporting information specifically to disazocondensation red pigments. For instance, you did not explain how mechanisms other than solubilisation through ionisation would -or would not- contribute to systemic toxicity, and how this allows a prediction of properties of the analogue substances. You also did not include a justification for the selection of the structurally similar pigments to exclude potential bias.
- you indicate your intention to perform static and dynamic dissolution assays to support the claims of poor absorption and low bioavailability, and to acquire the necessary supporting information with regard to your claims on bioavailability. You

³ Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

also state that, in absence of available guidance or fixed criteria for the assessment of lack of bioavailability, *"any toxicokinetic study potentially performed may be potentially considered as inadequate because the limit of detection or the investigated tissues are viewed as insufficient."*

First, the existing information gives some indications about low bioavailability based on molecular size and solubility. However, in the absence of data demonstrating absence of bioavailability (e.g. toxicokinetic studies), it is not possible to conclude on bioavailability for any of the analogue substances. Furthermore, the fact that the analogue substances PB 23 and PR 166 have been tested positive in ames tests only in presence of metabolic activation suggest that enzymatic metabolism can be relevant for the analogue substances tested. Your theoretical considerations on the absence of bioavailability have not been substantiated by experimental data such as studies on toxicity after repeated exposure (e.g. OECD TG 407/421/422), and are thus rejected.

Second, it is not possible to conduct an evaluation of the referred supporting information in absence of sufficient documentation and in the absence of an explanation of their relevance for your read-across adaptation.

Third, it is in your discretion to generate and provide the necessary supporting information in order to justify your read-across adaptation. If you do so, you are responsible for demonstrating the fulfilment of the requirements of Section 1.5 of Annex XI to REACH.

Bridging studies to compare such properties of the analogue substances

You did not provide appropriate bridging studies (such as a screening study OECD TG 421 or 422) to compare the properties of the analogue substances with regard to repeated dose and reproductive/developmental toxicity. As also explained in the next section (data density), your hypothesis of low bioavailability is not supported by results from repeated dose toxicity studies with representative analogue substances.

In the absence of such information, you have not established that the target and the source substances are likely to have similar properties. The allegation on potential metabolism is unsubstantiated, without further explanation on its impact on the prediction of hazardous effects by potentially bioavailable parent substance.

Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

2. Data density to derive a regular pattern

Annex XI, Section 1.5. provides that *"substances whose physicochemical, toxicological and eco-toxicological properties are likely to be similar or follow a regular pattern as result of structural similarity may be considered as a group or 'category' of substances."*

Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance⁴ and related documents^{5, 6}. To identify a regular pattern and/or to derive reliable prediction of the properties of the analogue substances, adequate and

⁴ Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals. 2008 (May) ECHA, Helsinki. 134. pp. Available online:

https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

⁵ Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: [Read-Across Assessment Framework \(https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across\)](https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)

⁶ Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017 (March) ECHA, Helsinki. 40 pp. Available online: <https://doi.org/10.2823/794394>

reliable information covering the range of structural variations identified among the analogue substances needs to be available.

You have provided the following valid studies:

1. For *in vitro* genotoxicity with bacteria (Annex VII, Section 8.4.1), two out of nine analogue substances (PR144 and PR166) have been tested according to OECD TG 471 with modified metabolic activation for azo-substances, such as the analogue substances, whereas all other bacterial tests are invalid because they are without this relevant modification.
2. For *in vitro* chromosomal aberrations in mammalian cells (Annex VIII, Section 8.4.2 and 8.4.3), one out of nine analogue substances (PR242) has been tested (OECD TG 473).
3. For *in vitro* gene mutations in mammalian cells, one out of nine analogue substances (PR166) has been tested (OECD TG 476).
4. For repeated dose toxicity (Annex VIII, Section 8.6.1 and Annex IX, Section 8.6.2), one category member (PR166) has been tested in an oral short-term (28-day) toxicity study (OECD TG 407, 2009) and one of the analogue substances (PR220) in a combined repeated dose toxicity and screening for reproductive / developmental toxicity (OECD TG 422, 2012). No repeated dose toxicity studies by the inhalation route have been provided. No sub-chronic toxicity studies (90-day) have been provided.
5. For screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1) and pre-natal developmental toxicity (Annex IX, Section 8.7.2), one of nine analogue substances (PR220) has been tested in a combined repeated dose toxicity and screening for reproductive/developmental toxicity (OECD TG 422, 2012). No pre-natal developmental toxicity studies have been provided.

Based on these studies you claim that there are similar properties between the analogue substances.

The analogue substances have multiple structural differences, but no information has been provided to establish whether and to what extent any of the analogue substances are representative of the whole category or a subset of it. In addition, the available studies cover only a small subset of these structural differences for each endpoint. Information for two (1.+4., above) or one (2., 3.+5., above) analogue substances is not sufficient to conclude which substances are representative of the analogue substances for *in vitro* genotoxicity, repeated dose toxicity and toxicity to reproduction and pre-natal development in the absence of (lower tier) toxicity studies with all analogue substances for the relevant endpoint. Considering the distinct structural differences between the analogue substances, there are too few data points (i.e. low data density) in the current data matrix for demonstrating consistency and predicting properties for the listed toxicological endpoints as proposed by you. Therefore, the information provided is not sufficient to conclude that toxicological properties are likely to follow a regular pattern.

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

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

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

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

1. Same *in vivo* genotoxicity study as request C.1 (triggered by Annex VII, Section 8.4., column 2)

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

You have provided the following studies in your dossier performed with the Substance:

- i. Key study: an OECD TG 471 study (██████████ 2006a).
- ii. Key study: an OECD TG 471 study (██████████ 2006b).
- iii. Key study: an OECD TG 471 study (██████████ 2006c).
- iv. Key study: an OECD TG 471 study (██████████ 2006d).
- v. Supporting study: a study similar to OECD TG 471 (██████████ 1980).
- vi. Supporting study: a study similar to OECD TG 471 (██████████ 1981a).
- vii. Key study: an OECD TG 476 study (██████████ 1989).
- viii. Key study: a study similar to OECD TG 474 (██████████ 1981b).
- ix. Supporting study: *in vivo* Sister Chromatid Exchange Assay (██████████ 1981c; 1990).
- x. Additional study: *in vivo* study to determine genotoxicity of mouse lymphoma cells (L5178Y) in host-mediated assay system (██████████ 1981d).

We have assessed this information and identified the following issue:

Trigger and information provided

A positive result in an *in vitro* gene mutation study in bacteria raises a specific concern for gene mutation that must be addressed including, where relevant, by further studies appropriate for that specific concern.

Your dossier contains positive results for the *in vitro* gene mutation studies in bacteria as listed above (██████████ 2006a; ██████████ 1980; ██████████ 1981a), which raise the concern for gene mutation. It also contains several other *in vitro* gene mutation studies in bacteria (██████████ 2006b; 2006c; 2006d) and mammalian cells (██████████ 1989) as well as *in vivo* studies (██████████ 1981b; 1981c, 1990; 1981d) with negative results.

However, the concern for gene mutation raised by the positive *in vitro* gene mutation tests in bacteria is not removed by the negative results in existing gene mutation tests because a positive dose-related increase in revertants was observed in different studies in the TA98 strain with metabolic activation, including one study following the Prival modification of the protocol recommended in OECD TG 471 for azo dyes and diazo compounds (██████████ 2006a) and two studies following the standard protocol (██████████ 1980; 1981a). Positive results were also consistently obtained in two studies in the TA1538 strain with metabolic activation without using the Prival modification (██████████ 1980; 1981a).

Some of the negative *in vitro* gene mutation tests in bacteria did follow the Prival modification of the protocol (██████████ 2006b; 2006c) and are considered valid, but the positive test results obtained with the same protocol, in the same testing conditions and by the same laboratory (██████████ 2006a) remain of concern without clear reasons for the result inconsistency.

These positive findings regarding gene mutation cannot be addressed by other *in vitro* tests (e.g., OECD TG 487 or 473), nor by other types of testing (e.g., toxicokinetic studies), which

do not provide any information related to the specific gene mutation concern raised by the positive *in vitro* gene mutation test *in bacteria*.

In addition, the existing *in vivo* studies are not addressing the gene mutation concern for the reasons described under Section C.1.

Therefore, the provided data are not appropriate for addressing the concern for gene mutation and are therefore this information requirement is not fulfilled.

Study selection and design

The Transgenic rodent somatic and germ cell gene mutation (TGR) and the comet assays are suitable tests to investigate and follow up the concern on gene mutation for the Substance. Other considerations, such as *in vitro* tests (e.g., OECD TG 487 or 473) or other types of testing (e.g., toxicokinetic studies) do not provide any information related to the specific gene mutation concern raised by the positive *in vitro* gene mutation test *in bacteria* and are, therefore, not appropriate.

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

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

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

1. Same in vivo genotoxicity study as request C.1 (triggered by Annex VII, Section 8.4., column 2)

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

This information requirement is triggered and not fulfilled by your registration dossier for the same reasons provided under Sections A.1.

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

2. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

An *In vitro* cytogenicity study in mammalian cells or an *In vitro* micronucleus study is a standard information requirement in Annex VIII to REACH.

You have adapted this information requirement by using a Grouping of substances and read-across approach under Annex XI, Section 1.5.

We understand that you have also submitted an adaptation based on a key study similar to OECD TG 474 (1981) on the Substance.

We have reviewed this information and identified the following deficiencies:

- a) As explained in the Appendix on general considerations, your adaptation based on a grouping of substances and read-across approach is rejected. Therefore, the information requirement is not fulfilled.
- b) Although you do not explicitly claim such an adaptation, ECHA understands that the information provided was submitted in order to meet the required information by way of adaptation under column 2 using existing, non-OECD, non-GLP studies. Such information must meet the requirements of Annex, Section XI 1.1.2. This rule enables registrants to claim that the data from experiments not carried out according to GLP or the test methods referred to in Article 13(3) can be considered equivalent to data generated by those test methods where a number of cumulative conditions are met, in particular:
 - Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case the OECD GD 474 includes the following key parameters: the proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes must be reported for each group of animals;

The reported data for the *in vivo* study you submitted did not include the reporting of the proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes for each group of animals.

Therefore, the provided *in vivo* test is not adequate, and your adaptation is rejected.

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

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

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

You have adapted the standard information requirement by using a Grouping of substances and read-across approach under Annex XI, Section 1.5.

As explained under Appendix General considerations, your adaptation according to Annex XI, Section 1.5 is rejected, and the information requirement is not fulfilled.

A study according to the test method OECD TG 421/422 should be performed in rats with oral⁷ administration of the Substance.

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

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

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

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

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

You have provided the following studies in your dossier performed with the Substance:

- i. Key study: an OECD TG 471 study (██████████ 2006a).
- ii. Key study: an OECD TG 471 study (██████████ 2006b).
- iii. Key study: an OECD TG 471 study (██████████ 2006c).
- iv. Key study: an OECD TG 471 study (██████████ 2006d).
- v. Supporting study: a study similar to OECD TG 471 (██████████ 1980).
- vi. Supporting study: a study similar to OECD TG 471 (██████████ 1981a).
- vii. Key study: an OECD TG 476 study (██████████ 1989).
- viii. Key study: a study similar to OECD TG 474 (██████████ 1981b).
- ix. Supporting study: *in vivo* Sister Chromatid Exchange Assay (██████████ 1981c; 1990).
- x. Additional study: *in vivo* study to determine genotoxicity of mouse lymphoma cells (L5178Y) in host-mediated assay system (██████████ 1981d).

We have assessed this information and identified the following issue:

In vivo study triggered

Your dossier contains several positive results for the *in vitro* gene mutation study in bacteria (██████████ 2006a; ██████████ 1980; ██████████ 1981a), which raise the concern for gene mutation as explained under Section A.1.

In your comments to the draft decision you argue that the positive findings in the *in vitro* study were obtained only in strains sensitive to aromatic amines, at precipitating concentrations, and in the absence of specific azo-reductive test conditions, suggesting a mutagenic effect due to an aromatic amine impurity. You also note you "*changed the boundary composition of the substance to exclude aromatic amine impurities to less than* ██████████

ECHA disagrees with your comments:

- You did not provide any evidence, even less demonstrated, that (1) the positive results are due to the impurities and (2) that such impurities of the test material are not representative of the Substance. Furthermore, the fact that the positive results were obtained in strains sensitive to aromatic amines and in the absence of specific azo-reductive test conditions might be indicative of the presence of such potentially mutagenic impurities but it does not exclude the possibility of mutagenic effects of the Substance itself.
- The studies were conducted according to OECD TG 471 that contains recommendations to test one or more precipitating concentrations for non-cytotoxic substances that are not soluble at the recommended maximum test concentration of 5 mg/plate or 5 µl/plate, and to test above 5 mg/plate or 5 µl/plate when evaluating substances containing substantial amounts of potentially mutagenic impurities. In addition, the

█ (2006a) Ames study used the Prival modification (i.e. azo-reductive test conditions) of the OECD TG 471 protocol.

- Furthermore, you have not characterised the test material impurities and it is therefore not possible to compare the test article with the Substance composition and the boundary composition.

Therefore, ECHA considers the positive Ames results obtained in the █ (2006a), █ (1980), and █ (1981a) studies valid and triggering an *in vivo* study, irrespectively of whether they are due to impurities or not.

No appropriate in vivo study

In order to adapt the information requirement, the *in vivo* somatic cell genotoxicity study must provide appropriate results and thus address the specific concern raised by the *in vitro* positive result.

You have provided an OECD TG 474 study and *in vivo* studies with negative results (█ 1981b; █ 1981c; 1990; █ 1981d).

The existing *in vivo* studies are not addressing the gene mutation concern raised by the *in vitro* data: the Nucleus Anomaly Test in Somatic Interphase Nuclei (█ 1981b) can only detect cytogenetic damage, not gene mutation, the *in vivo* Sister Chromatid Exchange Assay (█ 1981c; 1990) is only an indicator test detecting putative DNA lesions, which can be repaired and do not necessarily reflect actual gene mutations, and you indicated that the host-mediated assay system (█ 1981d) is of very low reliability.

Therefore, none of the *in vivo* data are appropriate to address the concern for gene mutation and the information requirement is not fulfilled.

Read-across adaptation

In your comments to the draft decision, ECHA understands that you have raised a read-across adaptation. You argue that the negative results obtained in *in vivo* studies with the Pigment Brown 23, using the same lot which led to positive results in the Ames test, are applicable for your substance. The available *in vivo* studies with Pigment Brown 23 are not addressing the gene mutation concern raised by the *in vitro* data: the *in vivo* Mammalian Erythrocyte Micronucleus Test (OECD TG 474) can only detect cytogenetic damage and the *in vivo* UDS test (OECD TG 486) is an indicator test detecting putative DNA lesions which should be used only when it can be reasonably assumed that the liver is a target organ, which is not the case here.

Furthermore, as explained under Appendix General considerations of this decision, your adaptation according to Annex XI, Section 1.5 is rejected. Therefore, your comments arguing the fact that, for Pigment Brown 23, negative results were obtained in *in vivo* studies with the same lot used in the Ames test giving positive results does not remove the concern for gene mutation for the Substance.

Test selection

The present decision requests the registrants concerned to generate and submit a reliable *in vivo* somatic cell study.

According to the ECHA Guidance Chapter R.7a⁸, the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation.

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

This decision, however, also requests an *in vitro* test under Annex VIII Section 8.4.2 (see Section B.2), which raises a concern for chromosomal aberration in case of positive results. The latter concern can be addressed by the comet assay, but not by the TGR assay. Considering the lower costs of the comet assay, the lower number of animals used and costs if one test is conducted rather than two, it is appropriate to wait for the results of the *in vitro* test requested under Annex VIII Section 8.4.2 and, depending on these results, to conduct either (1) the TGR or comet assay, or (2) the comet assay.

Test design

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

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

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

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

Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on

⁸ ECHA Guidance Chapter R.7a, Section R.7.7.6.3

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

Therefore, in case you decide to perform the comet assay, you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*⁹) in addition to the other aforementioned tissues, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells.

In case you decide to perform the TGR, you may consider to collect the male germ cells at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488 the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below $-70\text{ }^{\circ}\text{C}$). Following the generation and analysis of data on somatic cells, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the 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 your comments to the draft decision you argue that an *in vivo* genotoxicity study may be disregarded as invalid study if "*no systemic availability to the target cells was proven.*" As described above, the TGR or the comet assay include testing at the site of contact for potential genotoxic effects, as well as after systemic exposure. Both tests are considered suitable to follow up the concern on gene mutation for the Substance. The comet assay is also suitable to follow up a positive *in vitro* result for chromosomal aberrations at the site of contact, irrespective of demonstrated systemic availability.

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

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

You have adapted the information requirement according to Column 2 of Annex IX, Section 8.6.2. based on not significant systemic uptake and no indication of toxicity observed in a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test according to OECD TG 422 study with an analogue substance and in a sub-acute toxicity study according to OECD TG 407 with the Substance.

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

Annex IX column 2 adaptation not met

As provided in Annex IX, Section 8.6.2, Column 2, you may adapt the information requirement, provided you fulfil the following criteria, including:

- i. the Substance is not inhalable and
- ii. no evidence of absorption,
- iii. particularly if such a pattern is coupled with limited human exposure.

⁹ O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. *J. Vis. Exp.* (90), e51576, doi:10.3791/51576

You have not demonstrated that all criteria are met:

- i. The data provided in your dossier indicate that the Substance is inhalable (as discussed further below) and uses are reported that include spray application.
- ii. You did not demonstrate that there is no evidence of absorption. More specifically, the OECD TG 407 study with the Substance and the OECD TG 422 study with an analogue substance provide inconclusive information and cannot be considered as no evidence of absorption because the studies did not investigate toxicokinetic properties such as absorption. Furthermore, the submitted OECD TG 422 study was performed with analogue substance, and as explained under Appendix General considerations, your adaptation according to Annex XI, Section 1.5 is rejected. Therefore the OECD TG 422 study cannot be used to demonstrate that there is no evidence of absorption.
 - (i) Human exposure cannot be considered as limited because widespread uses, including professional and consumer uses are reported.

Therefore, your adaptation is rejected.

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

First, following the criteria provided in Annex IX, Section 8.6.2, Column 2, the inhalation route is the most appropriate route of administration to investigate repeated dose toxicity¹⁰. The sub-chronic toxicity study must be performed according to the OECD TG 413, in rats and with administration of the Substance by inhalation. The information provided in the technical dossier and the chemical safety report on properties of the Substance and its uses (industrial, professional and consumer uses, including PROCs 7 and 11 industrial and non-industrial spraying) indicate that human exposure to the Substance by the inhalation route is likely. More specifically, the Substance is reported to occur as a dust with a significant proportion of particles of inhalable size. Furthermore, the Substance is respirable [REDACTED] of low water solubility and consequently there is a potential for accumulation of the substance in the lungs. The test must be therefore performed by the inhalation route using the test method OECD TG 413.

Second, there is evidence that the lower respiratory tract is the primary site of deposition and retention of the Substance, because it is poorly soluble in water and respirable.

In your comments on the initial draft decision you state that "*The request for information on sub-acute toxicity via the inhalation route and based on current discussions on respirable, inert dusts and their regulation, the need for a clear definition and assessment criteria became overt*" and "*We consider organic pigments to belong to the group of poorly soluble, low toxicity materials (PSLT)*".

You are reminded that several paragraphs of the OECD TG 413 address specific issues related to testing of poorly soluble solid aerosols. Details on measurement and evaluation of lung burden are also provided in the OECD GD 39.

Further, you have not provided supporting evidence for your argument on any 'current discussions', on PSLT, nor a reason for how such grouping would affect this information requirement.

Therefore, you are requested to perform measurements of lung burden and bronchoalveolar lavage fluid (BALF) which are specifically designed to address such situation. The latest guidance on how to perform such measurements are described in the revised version of the OECD 413 test guideline adopted on 25 June 2018.

¹⁰ ECHA Guidance R.7a, Section R.7.5.4.3.

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

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

You have adapted the information requirement according to Column 2 of Annex IX, Section 8.7. based on lack of toxicity and absorption, and no significant human exposure of the general population.

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

Annex IX column 2 adaptation not met

According to Annex IX, Section 8.7., Column 2, third indent, the study does not need to be conducted if the substance is of low toxicological activity. This needs to be demonstrated with three concomitant criteria, including:

- i. that it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure; and
- ii. that there is no or no significant human exposure.

You have not demonstrated that all criteria are met:

- (i) You have not provided any toxicokinetic data to prove that no systemic absorption occurs. The OECD TG 422 and OECD TG 407 studies, or any provided studies, did not investigate toxicokinetic properties such as absorption.
- (ii) Furthermore, as discussed under section 2 of Appendix C, the uses of the Substance you identified indicate that there is possibility for significant human exposure.

Therefore, your adaptation is rejected.

OECD TG 422 study does not fulfill the requirement

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

You have not provided information following OECD TG 414. Instead, you have provided an OECD TG 422 study. In this study, structural malformations and variations are not investigated as required in the PNDT study (OECD TG 414). Therefore, this study does not fulfil the information requirement.

A PNDT study according to the test method OECD TG 414 should be performed in rat or rabbit as preferred species with oral¹¹ administration of the Substance.

¹¹ ECHA Guidance R.7a, Section R.7.6.2.3.2.

Appendix E: Procedural history

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

The compliance check was initiated on 17 January 2019.

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 within 30 days of the notification.

ECHA took into account your comments and did not amend the request(s) or the deadline.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix F: Observations and technical guidance

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

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 the Member States.

3. Test guidelines, GLP requirements and reporting

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

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

Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries'¹².

4. Test material

Selection of the test material(s)

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

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

Technical reporting of the test material

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

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"¹³.

5. List of references of the ECHA Guidance and other guidance/ reference documents¹⁴

¹² <https://echa.europa.eu/practical-guides>

¹³ <https://echa.europa.eu/manuals>

¹⁴ <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

Evaluation of available information

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

QSARs, read-across and grouping

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

ECHA Read-across assessment framework (RAAF, March 2017)¹⁵

Physical-chemical properties

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment

Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

OECD Guidance documents¹⁶

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.

Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment – No 43, referred to as OECD GD43.

¹⁵ <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

¹⁶ <http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>

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

Registrant Name	Registration number	(Highest) Data requirements to be fulfilled
[REDACTED]	[REDACTED]	[REDACTED]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.