Decision number: TPE-D-2114300120-80-01/F Helsinki, 27 May 2015

DECISION ON TESTING PROPOSAL(S) SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For Reaction mass of 2-(1,1-dimethylpropyl)anthraquinone and 2-(1,2-dimethylpropyl)anthraquinone, (List No 915-623-1), registration number: [redacted]

Addressee: [redacted]

The European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 51 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

I. Procedure

Pursuant to Article 40(1) of the REACH Regulation, ECHA has examined the following testing proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(e) thereof for Reaction mass of 2-(1,1-dimethylpropyl)anthraquinone and 2-(1,2-dimethylpropyl)anthraquinone, (List No 915-623-1), submitted by [redacted] (Registrant).

- Viscosity (OECD114) using the registered substance
- Long-term toxicity to aquatic invertebrates (OECD 211) using the registered substance
- Repeated dose toxicity: oral. 90-d (OECD 408) in rats, using the registered substance
- Genetic toxicity in vivo (Comet Assay)
- Genetic toxicity in vivo (EU Method B.12, OECD 474)
- Developmental toxicity/teratogenicity (OECD 414) in rats

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 1000 tonnes or more per year. This decision does not take into account any updates after 15 January 2015, the date upon which ECHA notified its draft decision to the Competent Authorities of the Member States pursuant to Article 51(1) of the REACH Regulation.

This decision does not imply that the information provided by the Registrant in his registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the registration at a later stage.

ECHA received the registration dossier containing the above-mentioned testing proposals for further examination pursuant to Article 40(1) on 2 May 2013.

ECHA held a third party consultation for the testing proposals covering sub-chronic toxicity (90-day):oral and genetic toxicity in vivo from 18 February 2014 until 4 April 2014. ECHA held a further third party consultation for the testing proposal covering reproductive toxicity (pre-natal developmental toxicity) from 15 April 2014 until 30 May 2014. ECHA received information from third parties (see section III below).

On 11 September 2014 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision.

On 13 October 2014 ECHA received comments from the Registrant agreeing to ECHA's draft decision.

The ECHA Secretariat considered the Registrant's comments. No amendments to the draft decision were made.

On 15 January 2015 ECHA notified the Competent Authorities of the Member States of its draft decision and invited them pursuant to Article 51(1) of the REACH Regulation to submit proposals for amendment of the draft decision within 30 days of the receipt of the notification.

Subsequently, proposal for amendment to the draft decision was submitted.

On 20 February 2015 ECHA notified the Registrant of the proposal for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposal for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposal for amendment received and did amend the draft decision.

On 2 March 2015 ECHA referred the draft decision to the Member State Committee.

By 23 March 2015, in accordance to Article 51(5), the Registrant provided comments on the proposal for amendment. The Member State Committee took the comments of the Registrant on the proposal for amendment into account.

A unanimous agreement of the Member State Committee on the draft decision was reached on 7 April 2015 in a written procedure launched on 26 March 2015.

ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Testing required

A. Tests required pursuant to Article 40(3)

The Registrant shall carry out the following proposed tests pursuant to Article 40(3)(a) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

1. Viscosity (Annex IX, Section 7.17.; test method OECD 114);
2. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: *Daphnia magna* reproduction test, EU C.20/OECD 211);
3. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: EU B.26/OECD 408) in rats;
4. *In vivo* mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2; test method B.12./OECD 474);
5. *In vivo* mammalian alkaline comet assay (Annex X, Section 8.4., column 2; OECD 489);
6. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414) in rats or rabbits, oral route.

Note for consideration by the Registrant:

The Registrant 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 of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring to and conforming with the appropriate rules in the respective Annex, and an adequate and reliable documentation.

Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, shall result in a notification to the Enforcement Authorities of the Member States.

B. Deadline for submitting the required information

Pursuant to Articles 40(4) and 22(2) of the REACH Regulation, the Registrant shall submit to ECHA by **5 June 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report. The timeline has been set to allow for sequential testing as appropriate.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposals submitted by the Registrant for the registered substance and scientific information submitted by third parties.

Tests required pursuant to Article 40(3)

1. Viscosity (Annex IX, Section 7.17.)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Viscosity" is a standard information requirement as laid down in Annex IX, Section 7.17. of the REACH Regulation. The information on this endpoint is not available for the registered substance subject to the present decision but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The Registrant has submitted a testing proposal for a Viscosity study according to OECD 114.

ECHA considers the proposed test appropriate and testing should be performed with the registered substance.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed test using the registered substance: Viscosity of liquids (test method: OECD 114).

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

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"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. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirement. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

The Registrant has submitted a testing proposal for the registered substance for long-term toxicity testing on aquatic invertebrates, *Daphnia magna* reproduction test, OECD 211 with the following justification: *"A long term toxicity test with aquatic invertebrates (section 9.1.5) is proposed, as the chemical safety assessment indicated the need to investigate further the effects on aquatic organisms as is required according to Annex IX of REACH. A daphnia magna reproduction study will be performed according to OECD guideline 211."*.

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 9.1.5 of the REACH Regulation.

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 1.2., November 2012), Chapter R7b (Section R.7.8.5 including Figure R.7.8-4), if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. There were no indications in the dossier from the short-term toxicity studies on aquatic species that the fish would be substantially more sensitive than aquatic invertebrates.
In such case, according to the integrated testing strategy, the *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study and the application of a relevant assessment factor no risks are observed (PEC/PNEC<1), no long-term fish testing may need to be conducted. However, if a risk is indicated, long-term fish testing may need to be conducted.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study using 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).

c) Notes for consideration by the Registrant

Once results of the proposed test on long-term toxicity to aquatic invertebrates are available, the Registrant shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation. If the revised chemical safety assessment indicates the need to investigate further the effects on aquatic organisms, the Registrant shall submit a testing proposal for a long-term toxicity test on fish in order to fulfil the standard information requirement of Annex IX, 9.1.6. If the Registrant comes to the conclusion that no further investigation of effects on aquatic organisms is required, he shall update his technical dossier by clearly stating the reasons for adapting the standard information requirement of Annex IX, 9.1.6.

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

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

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. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The Registrant has submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats via the oral route (OECD 408) with the following justification: *"Currently only a 28-day study is available with a reliability of 2 (e.g. no full report available; only 10 animals per dose level). To refine the DNEL values it is considered justified to propose a 90-day oral repeated dose toxicity study. This study is also important to refine the PNEC for secondary poisoning."*.

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.6.2. of the REACH Regulation .

The Registrant proposed testing by the oral route. In light of the properties of the substance and the information provided on the uses and human exposure, ECHA considers that testing by the oral route is most appropriate.

The Registrant proposed testing in rats. According to the test method EU B.26/OECD 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD 408).

4. *In vivo* mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex X, Section 8.4. provides that "*If there is a positive result in any of the in vitro genotoxicity studies in Annexes VII or VIII, a second in vivo somatic cell test may be necessary, depending on the quality and relevance of all the available data.*"

An appropriate second *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations is not available for the registered substance but may be necessary to meet the information requirements. Consequently there is an information gap and the Registrant proposed to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for an *In vivo* mammalian erythrocyte micronucleus test (EU Method B.12, OECD 474) with the following justification: *"According to Column 2 of Annex IX of REACH appropriate in vivo mutagenicity tests should be considered (section 8.4) in case of positive results of the genotoxicity tests in Annex VII and VIII. As the in vitro chromosome aberration study gave positive results, a testing proposal for an OECD 474 study is included."*.

ECHA notes that this test is an appropriate test to investigate effects on chromosomal aberrations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.1. and figure R.7.7-1 (February 2014).

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: *In vivo* mammalian erythrocyte micronucleus test (test method: EU B.12./OECD 474).

Notes for consideration by the Registrant

Due to the nature of the substance, the Registrant is reminded that, according to paragraph 10 of the OECD 474 (Mammalian Erythrocyte Micronucleus Test, updated on 26 Sept 2014) "If there is evidence that the test substance(s), or its metabolite(s), will not reach the target tissue, it may not be appropriate to use this test". Additionally, according to paragraph 48 (d) of the OECD 474, a test chemical is considered clearly negative if "Bone marrow exposure to the test substance(s) occurred". Accordingly, if a substance is negative in this test, and if it is not possible to demonstrate that bone marrow exposure to the substance occurred, then ECHA will consider any remaining uncertainty concerning the mutagenic potential of the substance and whether to request any further information.

Regarding follow up testing, the Registrant is reminded that according to the column 2 of section 8.4 of Annex IX of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "*the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered.*

5. *In vivo* mammalian alkaline comet assay (Annex X, Section 8.4., column 2)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex IX, Section 8.4. provides that "If there is a positive result in any of the in vitro genotoxicity studies in Annex VII or VIII and there are no results available from an in vivo study already, an appropriate in vivo somatic cell genotoxicity study shall be proposed by the Registrant."

An appropriate in vivo genotoxicity study to follow up the concern on gene mutations is not available for the registered substance but shall be proposed by the Registrant.

Consequently, there is an information gap and the Registrant proposed to generate information for this endpoint.

The Registrant proposed further in vivo testing according to OECD draft Guideline for in vivo mammalian alkaline comet assay in rodents for detection of DNA damage to follow the genotoxicity effects with the registered substance.

ECHA notes that this test is an appropriate test to investigate further effects on gene mutations and chromosomal aberrations in vivo as described in the ECHA Guidance document on information requirements and chemical safety assessment, chapter R.7.7.1. and table R.7.7-3 (August 2013). Strain E.coli WP2 uvr A tested negative, eliminating a possible cross-linking potential via this testing system. This is important information considering that one of the limitations of the comet assay (without modifications) is the low sensitivity to detect cross-linking agents.

The Registrant did not indicate the species or the route of exposure in his testing proposal. According to this test method, the rat is the default species. ECHA considers this species as being appropriate and testing should be performed with the rat.

As regards the route of administration, paragraph 39 of the draft OECD test guideline states "The anticipated route of human exposure should be considered when designing an assay" and "In any case the route should be chosen to ensure adequate exposure of the target *tissue(s)*". In light of the physicochemical properties of the substance, ECHA considers that testing by the oral route is appropriate.

ECHA notes that paragraph 42 of the draft OECD test guideline states "*The liver has been the tissue most frequently studied and for which there are the most data. Therefore, in the absence of any background information, and if no specific tissues of interest are identified, sampling the liver would be justified as this is a primary site of xenobiotic metabolism and is often highly exposed to both parent substance(s) and metabolite(s). In some cases examination of a site of direct contact (for example, for orally-administered substances the glandular stomach or duodenum/jejunum, or for inhaled substances the lungs) may be most relevant*". Therefore ECHA considers that the Comet assay should be performed in liver and either glandular stomach or duodenum/jejunum.

ECHA also points out that strain *E.coli* WP2 uvr A tested negative, eliminating a possible cross-linking potential via this testing system. This means that performance of the Comet assay can be considered as appropriate despite the low sensitivity of the Comet assay (without modifications) to detect cross-linking agents.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

The third party has proposed a strategy for ECHA to consider before further tests on animals are requested. However, third parties were invited, as specified by Article 40(2) of the REACH Regulation to submit "scientifically valid information and studies that address the relevant substance and hazard end-point, addressed by the testing proposal". As the proposal for a strategy as such cannot be regarded information or studies, ECHA concludes that this is not a sufficient basis to fulfil the data/information requirement.

Nevertheless, ECHA acknowledges that the Registrant may choose to use the information provided by the third party. The Registrant might consider combining the genetic toxicity studies but integration in the 90 days study is problematic. In this case an updated dossier containing the testing details must be provided.

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: *In vivo* mammalian alkaline comet assay in rat via the oral route (test method: OECD 489), with examination of liver and either glandular stomach or duodenum/jejunum.

Notes for consideration by the Registrant

The Registrant is reminded that according to the column 2 of section 8.4 of Annex IX of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered". ECHA notes that the examination of gonadal cells would optimize the use of animals. Positive results in whole gonad that contains a mixture of somatic and germ cells are not necessarily reflective of germ cell damage, but they indicate that tested substance(s) and/or its metabolites have reached the gonad and caused genotoxic effects. This type of evidence may still be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

The Member State proposed that the registrant should consider that in case of a negative test result of the *in vivo* micronucleus test, an *in vivo* gene mutation test should be performed to fully prove there is no mutagenic potential. In view of 3R, the registrant may consider to integrate the proposed *in vivo* micronucleus test with an in vivo comet assay[1,2,3].

6. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The Registrant has submitted a testing proposal for a pre-natal developmental toxicity study in rats according to EU B.31/OECD 414 with the following justification: "*In accordance with*

[1] Vasquez, M.Z. (2010). Combining the in vivo comet and micronucleus assays: a practical approach to genotoxicity testing and data interpretation. *Mutagenesis* 25 (2), 187-19.
[2] Recio L *et al*, (2010), Dose-response assessment of four genotoxic chemicals in a combined mouse and rat micronucleus (MN) and Comet assay protocol, *J. Toxicol. Sci.* 35:149-62.
[3] Bowen DE, *et al* (2011) Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the Comet assay and the flow-cytometric peripheral blood micronucleus test. *Mutat Res* 722: 7-19.

column 2 of REACH Annex IX, a teratogenicity study performed according to OECD guideline 414 is being proposed.".

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

The Registrant proposed testing in rats. He proposed testing by the oral route. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat or the rabbit as a first species to be used.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in rats or rabbits, oral route (test method: EU B.31/OECD 414).

c) Notes for consideration by the Registrant

In addition, a pre-natal developmental toxicity study on a second species is part of the standard information requirements as laid down in Annex X, Section 8.7.2. for substances registered for 1000 tonnes or more per year (see sentence 2 of introductory paragraph 2 of Annex X).

When considering the need for a testing proposal for a prenatal developmental toxicity study in a second species, the Registrant should take into account the outcome of the pre-natal developmental toxicity study on the first species and all available data to determine if the conditions are met for adaptations according to Annex X, Section 8.7. column 2, or according to Annex XI; for example if the substance meets the criteria for classification as toxic for reproduction Category 1B: May damage the unborn child (H360D), and the available data are adequate to support a robust risk assessment, or alternatively, if Weight of Evidence assessment of all relevant available data provides scientific justification that the study in a second species is not needed. If the Registrant considers that the conditions for adaptations are not fulfilled, he should include in the update of his dossier a testing proposal for a pre-natal developmental toxicity study on a second species. If the Registrant comes to the conclusion that the conditions for these adaptations can be fulfilled, he should update his technical dossier by clearly stating the reasons for proposing to adapt the standard information requirement of Annex X, Section 8.7.2. of the REACH Regulation.

IV. Adequate identification of the composition of the tested material

The process of examination of testing proposals set out in Article 40 of the REACH Regulation aims at ensuring that the new studies meet real information needs. Within this context, the Registrant's dossier was sufficient to confirm the identity of the substance to the extent necessary for examination of the testing proposal. The Registrant must note, however, that this information has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In addition, it is important to ensure that the particular sample of substance tested in the new studies is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually

manufactured. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Finally, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the studies to be assessed.

V. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Article 51(8) of the REACH Regulation. Such appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

[redacted]
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