EUROPEAN CHEMICALS AGENCY

Decision number: CCH-D-2114315619-46-01/F Helsinki, 30 March 2016

DECISION ON A COMPLIANCE CHECK OF A REGISTRATION PURSUANT TO ARTICLE 41(3) OF REGULATION (EC) NO 1907/2006

For N-isopropyl-N'-phenyl-p-phenylenediamine, EC No 202-969-7 (CAS No 101-72-4), 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 41(1) of the REACH Regulation ECHA has performed a compliance check of the registration for N-isopropyl-N'-phenyl-p-phenylenediamine, EC No 202-969-7 (CAS No 101-72-4), submitted by [redacted] (Registrant). The scope of this compliance check decision is limited to the standard information requirements of Annex VIII, Section 9.2.2.1 and Annex X, Section 8.4. of the REACH Regulation. ECHA stresses that it has not checked the information provided by the Registrant and other joint registrants for compliance with requirements regarding the identification of the substance (Section 2 of Annex VI).

This decision is based on the registration 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 6 February 2015, i.e. the date when the draft decision was notified to the Registrant under Article 50(1) of the REACH Regulation.

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

The compliance check was initiated on 31 July 2014.

On 6 February 2015 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 16 March 2015 ECHA received comments from the Registrant on ECHA's draft decision and expressed an intention to update the registration.

On 27 March 2015 the Registrant updated his registration dossier with the submission number [redacted]. ECHA notes that, as mentioned above, this decision does not take into account any updates after the date when the draft decision was notified to the Registrant. Any such update will be examined by ECHA only after the deadline established in the present decision has passed. ECHA also notes that the tonnage band of the joint submission remained at ≥ 1000 t after the update.

The ECHA Secretariat considered the Registrant's comments. The information is reflected in the Statement of Reasons (Section III) whereas no amendments to the Information Required (Section II) were made.

On 11 June 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, proposals for amendment to the draft decision were submitted.

On 17 July 2015 ECHA notified the Registrant of the proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

The ECHA Secretariat reviewed the proposals for amendment received and amended the draft decision.

On 27 July 2015 ECHA referred the draft decision to the Member State Committee.

By 17 August 2015, in accordance to Article 51(5), the Registrant provided comments on the proposals for amendment. In addition, the Registrant provided comments on the draft decision. The Member State Committee took the comments on the proposals for amendment of the Registrant into account. The Member State Committee did not take into account the Registrant's comments on the draft decision as they were not related to the proposals for amendment made and are therefore considered outside the scope of Article 51(5).

After discussion in the Member State Committee meeting on 15-17 September 2015, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 17 September 2015.

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

II. Information required

Pursuant to Articles 41(1), 41(3), 10(a)(vi) and/or (vii), 12(1)(e), 13 and Annexes VIII and IX of the REACH Regulation the Registrant shall submit the following information using the indicated test methods and the registered substance subject to the present decision:

1. Hydrolysis as a function of pH (Annex VIII, 9.2.2.1; test method: Hydrolysis as a function of pH, EU C.7/OECD 111); and

2. In vivo mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2; test method: EU B.12./OECD 474);

   in combination with

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

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, will result in a notification to the Enforcement Authorities of the Member States.

Pursuant to Articles 41(4) and 22(2) of the REACH Regulation the Registrant shall submit to ECHA by **6 April 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report.

III. Statement of reasons

Pursuant to Article 41(3) of the REACH Regulation, ECHA may require the Registrant to submit any information needed to bring the registration into compliance with the relevant information requirements.

Pursuant to Articles 10(a)(vi) and/or (vii), 12(1)(e) of the REACH Regulation, a technical dossier for a substance manufactured or imported by the Registrant in quantities of 1000 tonnes or more per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

1. Hydrolysis as a function of pH (Annex VIII, 9.2.2.1; test method: Hydrolysis as a function of pH, EU C.7/OECD 111)

The technical dossier contains data for this standard information requirement in the form of three study records.

Two of the study records contain information on hydrolysis which, according to the Registrant, is reliable as it meets Klimisch criterion 2. However, they contain information on hydrolysis at pH = 7 only. According to the test method used, namely Method C.7 in Commission Regulation (EC) No 440/2008, "The hydrolysis test should be performed at pH values of 4, 7 and 9" (Section 1.8.3 of the method).

The third study record contains information on hydrolysis measured at pH values of 4, 7 and 9. However, according to the Registrant, the information meets Klimisch criterion 4 only and is therefore not assignable. The Klimisch criterion is a system to assess the reliability of data as laid out in the Guidance on information requirements and chemical safety assessment Chapter R.4: Evaluation of available information, Section R.4.2 (Version of December 2011).

As the information reported in the technical dossier does not contain reliable data for all the values prescribed by the method, it does not fulfil the standard information requirement of Annex VIII, section 9.2.2.1 of the REACH Regulation.

In the comments to the draft decision made according to Article 50(1) the Registrant indicated a *"suitable and reliable study covering the endpoint hydrolysis"* will be made accessible and expressed an intention to update the registration with a robust summary of the study which would form part of a weight of evidence approach to cover the information requirement.

Therefore, the Registrant is requested to submit the following information derived with the registered substance subject to the present decision:

Hydrolysis as a function of pH (Annex VIII, 9.2.2.1; test method: Hydrolysis as a function of pH, EU C.7/OECD 111)

2. *In vivo* mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2; test method EU.B 12./ OECD 474) in combination with *In vivo* mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD 489) in rats by oral route, on the following tissues: liver and glandular stomach

"Mutagenicity" is an information requirement as laid down in Section 8.4. of Annexes VIII, IX and X to 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."

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 contains two *in vitro* studies, a chromosome aberration assay and a sister chromatid exchange assay, both performed according to the NTP standard protocol and with the registered substance. Both studies show positive results.

The positive results indicate that the substance induces chromosomal aberrations under the conditions of the tests.

However, the Registrant did not provide an appropriate *in vivo* somatic cell test to detect chromosomal aberrations. The three *in vivo* cytogenicity studies which have been provided are considered by ECHA as being not appropriate as the Registrant himself considers the studies as limited and not reliable.

The Registrant recognises the positive effects of the *in vitro* chromosome aberration assay (key study) and the *in vitro* sister chromatid exchange assay (supporting study) but considers the relevance of the results as being unclear. In particular, the Registrant considers the relevance of the *in vitro* chromosome aberration assay results are unclear because of the missing cytotoxicity data.

ECHA notes that this information was evaluated by an international body (OECD SIDS N- N-Isopropyl-N'-phenyl-p-phenylenediamine (IPPD), March 2000) with the recommendation that as "No information is available on the exact nature of the aberrations, however, it would be prudent regard this result as positive". Furthermore, it was concluded that "*In vitro* mammalian cell mutagenicity assays demonstrate IPPD has a potential to induce chromosome aberrations in the absence or presence of exogenous metabolic activation. The potential for direct acting genotoxicity was also demonstrated in a study of sister chromatid exchange. Negative results were obtained *in vitro* with respect to gene mutation and unscheduled DNA synthesis. No *in vivo* data are available."

A Member State proposed to amend the current test by combining the chromosomal aberration or micronucleus assay with a comet assay. The Member State expressed concerns that due to the substance's reactivity and liver weight increases seen in the repeated dose toxicity study, it is likely that the liver is the target organ and the substance never reaches the bone marrow. One of the strengths of the comet assay lies in its ability to detect DNA damage in other target organs than the bone marrow, such as liver or the site of first contact.

In his comments to the proposal for amendment, the Registrant stated that he prefers not to combine these two assays because the toxicity studies and their hematological parameters indicate that *the bone marrow is a potential target organ*. The Registrant further argued that, in any event, in the micronucleus assay *the number of immature erythrocytes/total erythrocytes will be recorded* which will tell whether the bone marrow was reached. In his final argument, the Registrant stated that combining the OECD TG 474 with a comet assay *would lead to substantially more complex study design and more animals to be used* because *an additional dose-finding study to define the precise dose or doses in the main experiment taking into account systemic and local effects* would be needed. The Registrant proposed to conduct an oral in vivo mammalian erythrocyte micronucleus test.

ECHA considers that the effects seen in the hematology parameters of the repeated dose toxicity study or possible changes seen in immature erythrocytes/total erythrocytes in a micronucleus assay may be caused by a toxic metabolite and not the parent compound, which seems to be of greater concern in terms of mutagenicity. With regard to the animal need, ECHA is of the opinion that the results of a dose-finding study for the micronucleus assay will apply for the comet assay as well and no additional animals will be needed.

Therefore, ECHA considers it necessary to perform an *in vivo* assay addressing chromosomal aberrations in combination with a comet assay, as described in the ECHA *Guidance* on information requirements and chemical safety assessment (version 3.0, August 2014), Chapter R.7a, Section R.7.7.1., Figure R.7.7-1. The Registrant can refer to Vasquez (2009)[1] and Bowen et al (2011)[2] for more detailed considerations when combining the comet assay with a micronucleus assay. Contrary to the OECD 475 the OECD 474 foresees the possibility to combine the tests.

[1] Vasquez,M.Z.(2010),Combining the *in vivo* Comet and micronucleus assays: a practical approach to genotoxicity testing and data interpretation, *Mutagenesis*, Vol. 25/2, pp. 187-99.

[2] Bowen, D.E. (2011), Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the Comet assay and the flow-cytometric peripheral blood micronucleus test, *Mutation Research*, Vol. 722/1, pp. 7-19.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 3.0, August 2014) Chapter R.7a, Table R.7.7-3, the *in vivo* mammalian erythrocyte micronucleus test (test method EU B.12 / OECD 474) is a test that identifies chemicals that induce structural and numerical chromosome aberrations and is therefore a suitable test to follow-up a positive result in an *in vitro* chromosomal aberration test. In the case of the registered substance, ECHA considers the *in vivo* mammalian erythrocyte micronucleus test (test method EU B.12 / OECD 474) as being appropriate. Comet assay recognises primary DNA damage that would lead to gene mutations and/or chromosome aberrations, but will also detect DNA damage that may be effectively repaired or lead to cell death.

According to the test method (EU B.12/OECD TG 474), the test shall be performed in mice or rats. Rat is considered the most appropriate because the repeated dose toxicity effects were seen in this species. Rat is also appropriate for the comet assay according to OECD 489. Considering the substance's physico-chemical properties performance of the test by the oral route is appropriate.

According to the test method (OECD TG 489), the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism and glandular stomach or duodenum/jejunum as site of direct contact. Because the substance is reactive, glandular stomach is considered the most appropriate site of direct contact.

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

In vivo mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2; test method: EU B.12./OECD 474)

in combination with

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

Notes for consideration by the Registrant

Micronucleus test

According to paragraph 10 of the OECD TG 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 TG 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.

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

Comet assay

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

IV. Adequate identification of the composition of the tested material

ECHA stresses that the information submitted by the Registrant and other joint registrants for identifying the substance has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation . The Registrant is reminded of his responsibility and that of joint Registrants to ensure that the joint registration covers one substance only and that the substance is correctly identified in accordance with Annex VI, Section 2 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 by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new studies must be suitable to assess these grades.

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 an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on 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.

Authorised[3] by Claudio Carlon, Head of Unit, Evaluation, E2.

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