EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114309734-50-01/F Helsinki, 20 October 2015

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

For 3,5-diamino-4-[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-2-[[2-sulfo-4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]benzoic acid sodium salt , EC No 480-890-9 (CAS No 906532-68-1), 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 proposal submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(c) thereof for 3,5-diamino-4-[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-2-[[2-sulfo-4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]benzoic acid sodium salt, EC No 480-890-9 (CAS No 906532-68-1), submitted by [redacted] (Registrant).

- *In vivo* mammalian alkaline comet assay (OECD 489), with oral administration, liver and site of contact (stomach or intestine) as target tissues.

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 10 to 100 tonnes per year. This decision does not take into account any updates after 8 April 2015, i.e. 30 calendar days after the end of the commenting period.

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.

The examination of the testing proposal was initiated upon the date when receipt of the complete registration dossier was confirmed on 6 August 2014, which included a testing proposal for the relevant endpoint.

ECHA held a third party consultation for the testing proposal from 18 November 2014 until 2 January 2015. ECHA did not receive information from third parties.

On 30 January 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. That draft decision was based on submission number [redacted].

On 4 March 2015 ECHA received comments from the Registrant on the draft decision. On 1 April 2015 the Registrant updated his registration dossier ([redacted]) changing the originally proposed test (*in vivo* mammalian erythrocyte assay in peripheral blood with the intravenous administration) to an *in vivo* mammalian alkaline comet assay by the oral route.

The ECHA Secretariat considered the Registrant's comments and update. On basis of this information, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

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 did amend 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. The Member State Committee took the comments of the Registrant on the proposals for amendment into account.

A unanimous agreement of the Member State Committee on the draft decision was reached on 31 August 2015 in a written procedure launched on 20 August 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 modified test pursuant to Article 40(3)(b) and 13(4) of the REACH Regulation using the indicated test method and the registered substance subject to the present decision:

1. *In vivo* mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD 489); which may be combined with an *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method: EU B.12./OECD 474); with oral administration. For the comet assay, the following tissues shall be analysed: liver and glandular stomach or duodenum/jejunum. If the Registrant opts for a combination with the micronucleus test, the bone marrow shall be analysed.

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.

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 **27 October 2016** 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

The decision of ECHA is based on the examination of the testing proposal submitted by the Registrant for the registered substance.

A. Tests required pursuant to Article 40(3)

1. *In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2)*; which may be combined with an *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2)

a) Examination of the testing proposal

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

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

Hence, initially the Registrant has submitted (submission [redacted]) a testing proposal for a *Mammalian Erythrocyte Micronucleus Test* with the following justification: [redacted] *was found to be positive in the bacterial reverse mutation assay, positive in the in vitro mammalian cell chromosomal aberration assay and positive in the in vitro mammalian cell gene mutation assay. In all three cases activation with rat and/or hamster S9 mix was necessary to reveal the genotoxic potential. This indicates that this substance is transformed by mammalian enzymes into one or more metabolites that can cause both gene mutations and chromosomal abnormalities. From the QSAR data, we cannot find similar structure in order to decide the mutagenicity. As a consequence, we propose in vivo gene mutation tests to verify the mutagenicity'.*

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations and chromosomal aberrations is not available for the registered substance but shall be considered. Consequently, there is an information gap and the Registrant considered it necessary to generate information for this endpoint.

Following ECHA's draft decision on 30 January 2015, the Registrant submitted comments indicating their intention to propose an alternative testing strategy to that originally submitted. In their subsequent dossier update (1 April 2015, submission [redacted]) the Registrant changed their testing proposal to the *in vivo* mammalian alkaline comet assay (test method: OECD 489) via oral route with the following rationale: *'In the kinetic assessment, the estimated oral adsorption of the testing substance is 10%. The systematic availability is low. OECD 489 comet assay test with oral administration could exam tissues*

at the site of contact, e.g. intestine or stomach. The substance does not need to pass through many organs before reaching targeted organs. The result would be reliable. Therefore, the comet assay test is chosen to verify the mutagenicity. Liver and site of contact (stomach or intestine) will be the target tissues'.

ECHA notes that the proposed test is an appropriate test to further investigate effects on gene mutations and 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 (August 2014). According to the ECHA Guidance on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3 (August 2014), the *in vivo* mammalian alkaline comet assay (OECD 489) detects 'primary DNA damage that would lead to gene mutations and/or chromosome aberrations' and is thus suitable to follow up positive result *in vitro* for gene mutation and chromosomal aberrations. Hence, ECHA considers this test to be most appropriate for the substance subject to the decision.

The Registrant proposed to perform the comet assay by the oral route to ensure adequate exposure of the target tissue(s).

As regards the route of administration, paragraph 39 of the OECD test guideline 489 states that "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 information provided on the uses and human exposure the substance is constituted of rather large particles (particle size distribution median: (D50) 35µm) and can be found in liquid form, with no spray application. Physico-chemical and toxicokinetic data show that the substance is highly soluble in water (>= 368 g/L, 20°C, pH 7.2), and there is systemic availability via oral route in spite of the large molecular weight (816.77 g/mol), and ECHA considers that testing by the oral route is appropriate.

ECHA notes that the Registrant did not indicate the test species. As regards the species to be used, paragraph 23 of the OECD test guideline 489 states that "the choice of rodent species should be based on (i) species used in other toxicity studies (to be able to correlate data and to allow integrated studies), (ii) species that developed tumours in a carcinogenicity study (when investigating the mechanism of carcinogenesis), or (iii) species with the most relevant metabolism for humans, if known. Rats are routinely used in this test." ECHA considers that testing in the rat is appropriate.

As regards the tissues to be studied, paragraph 42 of the draft OECD test guideline 489 states that "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 in glandular stomach or duodenum/jejunum.

ECHA notes the comments of the Registrant to the MSCA's Proposal for Amendment to suggest to the Registrant to integrate a micronucleus test with a comet assay, and his agreement to combine these two tests. The micronucleus test is suitable to investigate other potential mechanisms of genotoxicity such as clastogenicity and aneuploidy. Integrating the micronucleus test with the comet assay can help the Registrant reduce the number of tests performed and the number of animals used. The Registrant should take into

account the combination aspects such as dosing and sampling following the principles described in the literature (see OECD test guideline 489 and e.g. Bowen et al. 2011[1]). Hence, ECHA concludes that in view of the optimal use of animals the Registrant may consider to perform a comet assay combined with a micronucleus test.

The Registrant is reminded that this decision does not take into account any updates submitted after 8 April 2015. All the new information in the later update(s) of the registration dossier will however be assessed for compliance with the REACH requirements in the follow-up evaluation pursuant to Article 42 of the REACH Regulation.

b) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, the Registrant is requested to carry out the following study with the registered substance subject to the present decision:
In vivo mammalian alkaline comet assay (test method: OECD 489) in rats; which may be combined with an *in vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method: EU B.12./OECD 474); with oral administration. For the comet assay, the following tissues shall be analysed: liver and glandular stomach or duodenum/jejunum. If the Registrant opts for a combination with the micronucleus test, the bone marrow shall be analysed.

Notes for consideration by the Registrant

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 (EC) No 1272/2008.

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 study 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 study 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 study 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 study to be assessed.

[1] Bowen D.E. 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. Mutation Research, 722, 7-19.

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.

Authorised[2] by Guilhem de Seze, Head of Unit, Evaluation E1

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