ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: CCH-D-2114308982-46-01/F Helsinki, 30 September 2015

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

**For Condensation products of tall-oil fatty acids with diethanolamine and triethanolamine, CAS No 67784-78-5 (EC No 267-053-1), 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 41(1) of the REACH Regulation ECHA has performed a compliance check of the registration for Condensation products of tall-oil fatty acids with diethanolamine and triethanolamine, CAS No 67784-78-5 (EC No 267-053-1), submitted by [redacted] (Registrant). The scope of this compliance check decision is limited to the standard information requirement of Annex IX, Section 8.4. of the REACH Regulation.

This decision is based on the registration as submitted with submission number [redacted], for the tonnage band of 100 to 1000 tonnes per year. This decision does not take into account any updates after the deadline for updating (13 March 2015) communicated to the Registrant by ECHA on 4 February 2015.

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 9 August 2013.

On 31 July 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 22 August 2014 ECHA received comments from the Registrant on the draft decision.

The Registrant updated his registration after the expiry of the deadline for updating (13 March 2015) communicated to the Registrant by ECHA on 4 February 2015 and therefore too late in the decision making process for being considered. If still relevant, the dossier update will be considered by ECHA in line with its follow up process after the deadline established in the present decision has passed.

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

On 17 July 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 amended the draft decision.

By 17 August 2015 the Registrant did not provide any comments on the proposal for amendment.

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. Information required

**A. Information in the technical dossier derived from the application of Annexes VII to XI**

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

> *In vivo* mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2; test method: EU B.12./OECD 474) in mice or rats, oral route;
> or
> *In vivo* mammalian bone marrow chromosomal aberration test (Annex IX, Section 8.4., column 2; test method: EU B.11./OECD 475) in mice or rats, 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, will result in a notification to the Enforcement Authorities of the Member States.

**B. Deadline for submitting the required information**

Pursuant to Articles 41(4) and 22(2) of the REACH Regulation the Registrant shall submit to ECHA by **7 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

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)(d) of the REACH Regulation, a technical dossier for a substance manufactured or imported by the Registrant in quantities of 100 to 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to IX of the REACH Regulation.

> *In vivo* mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2)
> or
> *In vivo* mammalian bone marrow chromosomal aberration test (Annex IX, Section 8.4., column 2)

"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."

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 an *In vitro* Mammalian Chromosome Aberration Test performed according to OECD Guideline 473 with the registered substance that shows a positive result with an exposure duration of 21 hours without metabolic activation.

The Registrant considered the positive response as not relevant for *in vivo* situations as this statistically significant increase in the number of metaphases with aberrations *"was restricted to a cytotoxic concentration (250 µg/mL, relative mitotic index [redacted]%)"*. The Registrant further notes that *"there were no other statistically significant increases in chromosome aberrations in this study and no indications of mutagenicity in the other two in vitro genetic toxicity studies."*

However, ECHA observes that according to the OECD Guideline 473 (July 1997, paragraph 16), the highest tested concentration *"should show a significant reduction in degree of confluency, cell count or mitotic index, (all greater than 50%)."* A mitotic index of [redacted] % may therefore not be regarded as severe cytotoxic effect. Furthermore, ECHA notes that there are also indications for a concentration-related response in the experiment with exposure duration of 21 hours and that the Registrant did not provide vehicle control data of the respective laboratory in the dossier.

The Registrant's reference to the negative results obtained in the two other genetic toxicity studies (Ames and *In Vitro* Mammalian Cell Gene Mutation Test) is not considered as a valid argument because these two studies aim to detect point mutations and gene mutations, whereas the *in vitro* chromosomal aberration study addresses structural chromosome aberrations.

ECHA therefore considers the result of the *In vitro* Mammalian Chromosome Aberration Test as a positive result inducing chromosomal aberrations under the conditions of the test.

ECHA received the Registrant's comments to the draft decision arguing that performance of an *in vivo* genotoxicity study is not considered necessary to determine the absence of a genotoxic potential of WS400128 because in vitro chromosomal aberration test "positive result is an artifactual, false positive result that can be disregarded".

The Registrant explained in its comments to the draft decision that "it is rather likely that micelles of the test substance were present at all concentrations used in the in vitro tests although turbidity or precipitates were not observed" and further referred to the in vitro mouse lymphoma study provided in the registration dossier where precipitate was indeed reported to be observed by eye in the end of every experiment and treatment (3 h and 24 h exposure times) with ethanol [redacted]% v/v final concentration in the medium. The Registrant also refers in its comments to draft version of the updated *In vitro* Mammalian Chromosome Aberration Test (OECD 473) guideline (published in 26 September 2014) according to which "care should be taken in interpreting positive results only to be found in the higher end of the cytotoxicity range of the defined cytotoxicity range" and that "the situation of false positive results due to (exaggerated) cytotoxicity or precipitates has been discussed extensively (e.g. [redacted])." The Registrant also provided as part of its comments the historical data for 30 continuous treatment experiments (21 h) between April 2007 and March 2009 claiming furthermore that, based on the historical data, "the number of aberrant cells detected in the present study are just at the upper level of the solvent control." Finally, the Registrant indicated in its comments that the registered substance is a condensation product and that the starting materials are well characterised and not classified for genotoxicity.

Firstly, ECHA notes that no observed precipitation for the given test is reported. Furthermore, the comparison of the test conditions of the in vitro Mammalian Chromosome Aberration Test and the provided *in vitro* mouse lymphoma study is not possible as the ethanol concentration used in *In Vitro* Mammalian Chromosome Aberration Test is not reported. Therefore, in ECHA's view speculating the presence and/or role of the precipitate based on the current information of the test conditions is inconclusive.

Secondly, ECHA notes that, according to OECD testing guideline for In vitro Mammalian Chromosome Aberration Test (OECD 473), the reported cytotoxicity (relative mitotic index [redacted]%) with the positive result in 21 hour experiment can be regarded as higher end of the cytotoxicity but not excessive. The final updated OECD 473 testing guideline (adopted 26 September 2014) states that "if the maximum concentration is based on cytotoxicity, the highest concentration should aim to achieve 55 ± 5% cytotoxicity using the recommended cytotoxicity parameters (i.e. reduction in RICC and RPD for cell lines and reduction in MI for primary cultures of lymphocytes to 45± 5% of the concurrent negative control). Care should be taken in interpreting positive results only to be found in the higher end of this 55 ± 5% cytotoxicity range."

Thirdly, ECHA notes that the relevant endpoint is "frequency of aberrant metaphases excluding gaps". The reported results in the registration dossier for the 21 h experiment show a concentration-related response, with frequency of aberrant metaphases excluding gaps of 1.5, 3.0, 4.0 and 6.0 for test concentrations of 0, 220, 235 and 250 µg/mL, respectively. Furthermore, the historical data for this endpoint, as provided by the Registrant in its comments (without S9 mix), are mean of [redacted]% with standard deviation of [redacted]% and [redacted]% upper confidence limit of 3.0. Therefore, ECHA concludes that the mean % of aberrant cells without gaps of 6.0 is above the solvent control [redacted]% confidence limits.

Finally, ECHA acknowledges the Registrant's evaluation of the genotoxic potential of the starting materials indicating no concern for genotoxicity for those starting materials. However, ECHA notes that the registered substance, which is a UVCB substance, might have properties that are different from the starting materials. Therefore, the information on genotoxicity from the starting materials cannot be used to disregard an effect that was observed with the condensation product.

ECHA concludes that the provided *in vitro* mammalian chromosome aberration test result is evaluated as "positive" in IUCLID under "Test results". Only in the "Applicant's summary and conclusion" this result is indicated as "ambiguous". After taking into account the Registrant's comments and the information made available, ECHA considers the Registrant did not provide a convincing justification for deviating from the conclusion of the study report "Test results" and therefore considers the result of the study as "positive".

Therefore, ECHA considers it necessary to perform an *in vivo* assay addressing chromosomal aberrations, as described in the ECHA *Guidance* on information requirements and chemical safety assessment (version 2.4, February 2014), Chapter R.7a, Section R.7.7.1. and Figure R.7.7-1.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 2.4, February 2014) Chapter R.7a, Table R.7.7-3, the *in vivo* mammalian bone marrow chromosome aberration test (test method EU B.11 / OECD 475) or the *in vivo* mammalian erythrocyte micronucleus test (test method EU B.12 / OECD 474) are tests that identify chemicals that induce structural and numerical chromosome aberrations and are therefore suitable tests to follow-up a positive result in an *in vitro* chromosomal aberration test.

In light of the physicochemical properties of the substance, ECHA considers that testing by the oral route is appropriate.

The Registrant is reminded that this decision does not take into account any updates submitted after 13 March 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.

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 (test method: EU B.12./OECD 474) in mice or rats, oral route
or
*In vivo* mammalian bone marrow chromosomal aberration test (test method: EU B.11./OECD 475) in mice or rats, oral route.

Notes for consideration by the Registrant

According to paragraph 10 of the OECD TG 474 (Mammalian Erythrocyte Micronucleus Test, updated on 26 Sept 2014) and to paragraph 6 of the OECD TG 475 (Mammalian Bone Marrow Chromosomal Aberration 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 and to paragraph 44 (d) of the OECD 475, 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 IX, 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".

The Registrant may consider making a testing proposal to conduct the mammalian spermatogonial chromosome aberration test (OECD TG 483) whenever the results of the somatic *in vivo* genotoxicity tests indicate that chromosomal aberrations occurred.

## IV. Adequate identification of the composition of the tested material

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 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 by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new study 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 study 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[1] by Claudio Carlon, Head of Unit, Evaluation E2

[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.