EUROPEAN CHEMICALS AGENCY

Helsinki, 12 December 2016

Addressee [redacted]

Decision number: TPE-D-2114349665-39-01/F
Substance name: Formaldehyde, reaction products with ethylenediamine
EC number: 281-928-5
CAS number: 84066-92-2
Registration number: [redacted]
Submission number: [redacted]
Submission date: 06.11.2015
Registered tonnage band: 100-1000 tpa

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA has taken the following decision.

While your originally proposed test for an in vivo mammalian erythrocyte micronucleus test (OECD TG 474) using the registered substance is rejected, you are requested to perform:

> 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, using the registered substance.

You 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 and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **19 December 2017.** You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

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, shall 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.

Authorised[1] by Kevin Pollard, Head of Unit, Evaluation E1

[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 1: Reasons

The decision of ECHA is based on the examination of the testing proposal(s) submitted by you.

In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2)

Pursuant to Article 40(3)(d) and (c) of the REACH Regulation, ECHA may reject a proposed test and require the Registrant to carry out other tests in cases of non-compliance of the testing proposal with Annexes IX, X or XI.

"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".

The technical dossier contains three *in vitro* studies (Ames test, [redacted]; in vitro mammalian chromosome aberration test in human lymphocytes, [redacted]; mouse lymphoma assay in L5178Y cells, [redacted]) performed according to GLP and OECD TGs 471, 473 and 476, respectively, with the registered substance. Both the chromosomal aberation test and the mouse lymphoma assay show clear positive results. In the *in vitro* chromosomal aberration assay, a high increase in the aberration rates was observed with and without metabolic activation. In the *in vitro* gene mutation assay, a strong positive effect with and without metabolic activation was observed. The potency of the effect was at the same level as the positive controls. The effect was caused mainly by the increase in the small colonies. Nevertheless an increase of large colonies was also observed. The positive *in vitro* results indicate that, although the potential to induce gene mutation cannot be ruled out, the main concern for this substance is chromosomal aberration.

An appropriate *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations is not available in the dossier for the registered substance. Consequently, there is an information gap and you proposed to generate information for this endpoint. Hence, you have submitted a testing proposal for a Mammalian Erythrocyte Micronucleus Test according to OECD Guideline 474 to be performed with the registered substance.

ECHA notes that the proposed test is an appropriate test to investigate effects on chromosomal aberrations *in vivo,* as described in the ECHA *Guidance on information requirements and chemical safety assessment* (version 4.1, October 2015), Chapter R.7a, section R.7.7.1. and figure R.7.7-1, if the test substance or its metabolite(s) will reach the target tissue as specified in the respective test method OECD TG 474.

ECHA further observes that there is currently no information on the potential aneugenicity of the substance subject to this decision in the technical dossier. According to the ECHA *Guidance document on information requirements and chemical safety assessment,* Table R.7.7-3 (version 4.1, October 2015), the *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474) is adequate to measure structural and numerical chromosome aberrations and has thus the potential to detect clastogenic and aneugenic effects.

This test is suitable to adequately further investigate the findings obtained in the *in vitro* mammalian chromosome aberration test performed according to the OECD 473 test guideline included in the technical dossier. Moreover, the *in vivo* mammalian erythrocyte micronucleus test may also provide additional information on an aspect of genotoxicity not yet explored for this substance. Specifically, in the revised OECD 474 test guideline, published on 26 September 2014, paragraph 42 mentions protocol adaptations (i.e. protocol including the use of a chromosome centromere labeling method, e.g. FISH, CREST) that may enable the determination of the mechanism of micronucleus induction and allow the distinction between clastogenic and aneugenic effects.

However, ECHA notes the following available data set for the registered substance:

- the potential for irritation and classification as Eye Irritant Category 2, H319, along with the classification as Skin Sensitizer Category 1B, which are indications of reactivity,
- the effects in stomach in the acute oral and the 28-day oral toxicity study in rats where blood and lesions in stomach were observed, respectively.

ECHA thus considers that there is a concern regarding potential mutagenic effects at the first site of contact. These mutagenic effects at the first site of contact can be addressed neither by the micronucleus test nor by the chromosomal aberration test because these tests are not investigating any site of contact tissues. According to the *ECHA Guidance on information requirements and chemical safety assessment* R.7a, chapter R.7.7.6.3 (version 4.1, October 2015), the *in vivo* mammalian alkaline comet assay (OECD TG 489) is suitable to follow up positive results *in vitro* showing gene mutation or chromosomal aberration assays. Therefore, this test is also suitable to adequately follow up the findings obtained in the *in vitro* mammalian chromosome aberration test performed according to the OECD TG 473 test guideline and the *in vitro* mammalian cells gene mutation test in L5178Y cells performed according to the OECD TG 476 test guideline included in the technical dossier. Moreover, the *in vivo* mammalian alkaline comet assay enables the generation of information regarding the potential genotoxic effects caused in several tissues, in particular in the site of contact tissue(s) which is not covered by the other test guidelines.

Initially, ECHA aimed at providing you the choice to perform either the micronucleus test or the comet assay. In your comments on that draft decision, you informed of your intention "to conduct the *in vivo* mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2; test method: EU B.12./OECD TG 474) in mice or rats, oral route using the registered substance, following ECHA's final decision".

However, ECHA received from Member State competent authorities two proposals for amendments to reject the testing proposal for the mammalian erythrocyte micronucleus test and to replace it by *in vivo* mammalian alkaline comet assay via the oral route because of the already mentioned high reactivity of the substance and the risk that a mutagenic effect is missed because the substance does not reach the target tissue in an erythrocyte micronucleus test. In the light of these proposals for amendment, ECHA considers that the comet assay is more appropriate to clarify the concern identified in *in vitro* tests, in particular to investigate the potential genotoxic effect at the site of first contact. Therefore, ECHA has modified the request to an *in vivo* mammalian alkaline comet assay and rejects your testing proposal which is not the most appropriate test to be performed for the registered substance.

You did not specify the species to be used for the proposed testing. Moreover, you did not specify the route for testing.

According to the test method OECD TG 489, the test shall 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.

According to the test method OECD TG 489, the test shall be performed by analysing three tissues: liver as primary site of xenobiotic metabolism; glandular stomach and duodenum as sites of contact. In respect of analysing the tissues of the gastro-intestinal tract, 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 sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the modified study with the registered substance subject to the present decision :

In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route **,** on the following tissues: liver, glandular stomach and duodenum.

Your originally proposed test for an *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) is rejected according to Article 40(3)(d) of the REACH Regulation.

Notes for your consideration

You are 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".

You may consider examining gonadal cells in addition to the other aforementioned tissues, 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 a 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.
Moreover, in case the *in vivo* comet assay on somatic cells is positive, you may consider making a testing proposal to conduct the mammalian spermatogonial chromosome aberration test (OECD TG 483).

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal(s) for examination pursuant to Article 40(1) on 6 November 2015.

ECHA held a third party consultation for the testing proposal(s) from 16 May 2014 until 30 June 2014. ECHA did not receive information from third parties.

This decision does not take into account any updates after **6 July 2016**, 30 calendar days after the end of the commenting period.

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.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the request(s).

ECHA notified the draft decision to the competent authorities of the Member States for proposal(s) for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-50 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your 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.

2. 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.

3. In carrying out the test(s) required by the present decision it is important to ensure that the particular sample of substance tested 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 or imported. If the registration of the substance covers different grades, the sample used for the new test(s) must be suitable to assess these. Furthermore, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.