EUROPEAN CHEMICALS AGENCY

Helsinki, 7 November 2016

Addressee: [redacted]

Decision number: TPE-D-2114347326-49-01/F
Substance name: N-{2-[(phenylcarbamoyl)amino]phenyl}benzenesulfonamide
EC number: 806-543-7
CAS number: 215917-77-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 14 September 2015
Registered tonnage band: 10-100T

DECISION ON A TESTING PROPOSAL

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

Your testing proposal is accepted and you are requested to carry out:

In vivo mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method: EU B.12./OECD TG 474) in mice or rats, oral route using the registered substance;

OR

In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum with 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 **14 November 2017**. You shall also update the chemical safety report, where relevant.

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 Ofelia Bercaru, Head of Unit, Evaluation E3

[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 above decision is based on the following considerations.

In vivo mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2)

OR

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

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 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."

The technical dossier contains an *in vitro* mammalian chromosome aberration test performed according to OECD Guideline 473 with the registered substance that shows positive result. You considered that the positive response was "*only slightly over*" the threshold for the number of structurally aberrant cells. Hence you considered necessary to examine the applicability of the positive response and to see if the effect is replicated *in vivo*. The positive result indicates that the substance is inducing chromosomal aberrations under the conditions of the test. Mutagenicity of the registered substance was also assessed in a bacterial reverse mutation test performed according to OECD Guideline 471. The test result for gene mutation was negative.

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

Hence, you have submitted a testing proposal for an *In vivo* mammalian erythrocyte micronucleus test.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Mutagenicity in vivo. ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement for which testing is proposed. ECHA has taken these considerations into account.

ECHA notes that the proposed test is an appropriate test to further 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 (EU B.12/OECD TG 474).

Alternatively, the *in vivo* mammalian alkaline comet assay ("Comet Assay", OECD TG 489) is a suitable test to be performed. 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 489) is suitable to follow up positive result *in vitro* showing gene mutation or chromosomal aberration. 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 473 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).

Therefore, ECHA provides you the choice to perform either the micronucleus test or the comet assay.

You did not specify the species to be used for testing. You did not specify the route for testing.

In case you decide to perform a micronucleous assay according to the test method (EU B.12/OECD TG 474), the test shall be performed in mice or 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.

In case you decide to perform the comet assay 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. The test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. 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)(a) of the REACH Regulation, you are 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 TG 474) in mice or rats, oral route;

or

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

Notes for your consideration

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 it may not be an appropriate test to meet the information requirements under the REACH Regulation and ECHA will consider any remaining uncertainty concerning the mutagenic potential of the substance and whether to request any further information.

Considering the above and in view of the 3Rs principle (replacement, reduction, refinement of experimental studies in vertebrate animals), you may consider to combine the proposed *in vivo* micronucleus test with an *in vivo* comet assay[2,3,4].

You are reminded that according to 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, for substances that give positive results in an in vivo test for genotoxic effects in somatic cells, "the potential for substances that give positive results in in vivo tests for genotoxic effects in somatic cells to affect germ cells should always be considered. The first step is to make an appraisal of all the available toxicokinetic and toxicodynamic properties of the test substance." Further, "if the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation will be necessary. In the event that additional information about the toxicokinetics of the substance would resolve the problem, toxicokinetic investigation (i.e. not a full toxicokinetic study) tailored to address this should be performed."

In case you decide to perform the micronucleous assay, and the results of the somatic *in vivo* genotoxicity tests indicate that chromosomal aberrations occurred you shall consider the need to make a testing proposal to conduct a mammalian spermatogonial chromosome aberration test (OECD TG 483).

In case you decide to perform the comet assay, 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 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.

[2] 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.
[3] 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.
[4] 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.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposal(s) for examination pursuant to Article 40(1) on 18 September 2015.

ECHA held a third party consultation for the testing proposal(s) from 30 November 2015 until 15 January 2016. ECHA did not receive information from third parties.

This decision does not take into account any updates after **27 April 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.

ECHA did not receive any comments by the end of the commenting period.

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.