ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 5 July 2019

Addressee: [redacted]

Decision number: TPE-D-2114475941-40-01/F
Substance name: Aluminium oxide
EC number: 215-691-6
CAS number: 1344-28-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 23/10/2017
Registered tonnage band: Over 1000

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposal(s) and decided as follows.

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

1. **In vivo mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2; test method: OECD 474) combined with in vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD 489), in rats, oral route with the two nanoforms of the registered substance described by the Registrant. For the micronucleus test, the bone marrow or peripheral blood, for the comet assay, liver, glandular stomach and duodenum shall be analysed. ECHA considers that it is at your discretion to perform 1) the micronucleus analysis both in bone marrow and in blood, 2) the comet assay also in blood and 3) the proposed additional toxicokinetic study during the testing program.**

You have to submit the requested information in an updated registration dossier by ***12 January 2021***. You shall also update the chemical safety report, where relevant.

The reasons for this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3. More specifically, the test material shall be reported in accordance with the prescription set out in Appendix 3.

On 3 December 2018, the European Commission adopted Commission Regulation (EU) 2018/1881 amending of the REACH Regulation by introducing notably new information requirements specific to nanomaterials in Annexes VI to XI. The revised requirements will enter into mandatory application on 1 January 2020 for all registrations. After that date, ECHA may examine your registration in accordance with Article 41(1) of the REACH Regulation, in order to verify that the information in your dossier complies with the requirements set out in the revised annexes. The present decision is without prejudice to future requests to submit any information needed to bring the registration into compliance

with the revised information requirements.

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, has to 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 Claudio Carlon, Head of Unit, Hazard Assessment

[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 and scientific information submitted by third parties.

1. **In vivo mammalian erythrocyte micronucleus test (Annex X, Section 8.4., column 2) combined with an in vivo mammalian alkaline comet assay (Annex X, 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 Section 8.4. of Annexes VII to X of the REACH Regulation. Column 2 of Annex X, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annexes VII or VIII, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data."

The technical dossier contains several *in vitro* studies *(micronucleus-, chromosomal aberration- and comet assay)* performed similarly to OECD TG 487, TG 473 and non guideline protocols all with analogue substances (aluminium sulphate, aluminium chloride, aluminium hydroxide, aluminium trichloride and aluminum trihydroxide) that show positive results. The dossier furthermore, contains several non GLP *in vivo* studies (publications) with the registered substance that show positive results:micronucleus test, chromosomal aberration test and comet assay, (Balasubramanyam et al. 2009a, 2009b) that were respectively performed according to OECD TG 474, 475 and the protocol described in Tice et al. 2000. The positive results indicate that the substance is inducing gene mutations and/or chromosomal aberrations under the conditions of the tests. However, regarding the studies of Balasubramanyam et al. (2009a, 2009b) you claim that there is missing characterisation of the test material: *"The material is described as > 90% aluminium oxide, but with no information about the remaining constituents or impurities. A characterisation of the so called nano-material according to current standards of test material characterisation for nano-materials is also missing".* Furthermore, you state that *"the test material used for the non GLP in vivo tests is not available on the market, is not a commercial product, and there is no way of characterising this material retrospectively".* You continue with expressing that *"Further to this we have severe doubts about the quality of this study and for that purpose have asked an independent expert* [redacted] *to review the publications".* You have enclosed the assessment of [redacted] in the technical dossier, in which he concludes: [...] *"However, the data are so perfect they raise concerns. Such clear dose-responses for different endpoints over multiple sampling times are rarely seen following a single administration, and cannot be easily explained. The standard deviations, at least for the MN scores, are consistently to low to be credible taking normal inter-replicate and inter-animal variability into account".*

In a proposal for amendment (PfA), submitted by one of the Member States Competent Authorities (MSCAs), it was indicated that the test materials used in the Balasubramanyam *et al.* (2009a, 2009b) studies are not well characterised and might contain relatively high amount of impurities which are not representative of the two nanoforms proposed for testing. Indeed, ECHA considers that the characterisation of the test materials used in the Balasubramanyam *et al.* (2009a, 2009b) studies is limited. The data provided only refers to

the particle size (30 and 40 nm), the shape (spherical) and the particle size distribution. Moreover, the impurities present in the test materials are not known/reported. According to the study data, the purity of the tested nanomaterials is higher than 90%, therefore a relatively high amount of impurities may potentially be present in the test materials.

In your comments on the PfA you stated that it has not been possible to obtain a sample of the test materials used in these studies. Therefore, you concluded that the sample used for the studies was a one off test batch which was never put in production.

In view of the above, an appropriate second *in vivo* genotoxicity study to follow up the concern on gene mutations / chromosomal aberrations is not available for the registered substance but may be necessary, depending on the quality and relevance of all the available data, to meet the information requirements.

ECHA notes that the particle size of the two nanoforms proposed by you to be tested and characterised/described in your dossier have a size of approximately 7 nm with relatively small particle size distribution compared to the nanomaterials tested in the Balasubramanyam *et al.* (2009a, 2009b) studies. This difference in particle size may potentially have an impact on the test results. Therefore, it is clear that the test materials used in the Balasubramanyam *et al.* (2009a, 2009b) studies may not be representative of the registered substance.

ECHA considers that a second *in vivo* somatic cell test is necessary considering that the available *in vivo* studies may not be relevant to 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 combined micronucleus (MN) and comet assay according to OECD TG 489 with the following justification: *"In order to confirm or refute the findings published by Balasubramanyam et al, it is recommended that a new combined MN and comet assay be performed, with oral dosing, to a robust OECD protocol. MN can be measured in bone marrow, blood or both. Comets should be measured in blood (so as to check the published findings) but also in the default tissues of GI tract (duodenum) and liver as recommended in the OECD guideline (OECD 2014c). The study should include concurrent negative and positive controls, toxicokinetics (to determine systemic exposure to aluminium), and concurrent cytotoxicity measures (including histopathology of duodenum and liver).*

It is our conclusion that an in vivo study as described is necessary for the correct assessment of aluminium oxide nano materials" to be performed with *"two commercial samples of nanoscale aluminium oxide for the test, that will be characterized according to the current state of the science".* You furthermore specify that *"The test is planned to be performed with two samples A and D as described below and characterized in the attached reports (substance characterization incl. granulometry, density, dustiness)".*

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity 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(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA notes that the proposed combined test is an appropriate test to further investigate effects on gene mutations and 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).

You proposed testing in rats by the oral route.

According to the test method 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.
You also propose that "*MN can be measured in bone marrow, blood or both*". ECHA considers that according to OECD TG 474 either the bone marrow or peripheral blood cells of animals can be used for analysing micronuclei at appropriate time(s) after treatment. ECHA considers that it is at your discretion to measure MN both in bone marrow and in blood.

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.
You propose that "*comets should be measured in blood (so as to check the published findings) but also in the default tissues of GI tract (duodenum) and liver*".
In line with the test method OECD TG 489, 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 analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract. ECHA considers that it is at your discretion to perform the analysis of comets also in blood.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

ECHA acknowledges that the third party has proposed a weight of evidence approach for you to consider.

ECHA notes that it is your responsibility to consider and justify any adaptation of the information requirements in accordance with the relevant conditions as established in Annex XI, Section 1.2. Therefore, you may assess whether you can justify weight of evidence as suggested by the third party. If the information requirement can be met by way of adaptation, you may include the adaptation argument with all necessary documentation according to Annex XI, Section 1.2. in an updated registration.

ECHA notes that the information provided by the third party is insufficient for demonstrating that the conditions of Annex XI, Section 1.2.of the REACH Regulation are met. For example, the third party has provided some literature data in a weight of evidence approach. Out of the 8 references provided, 6 are related to *in vitro* genotoxicity, one (Park et al. 2015) on general toxicity without special information on genotoxicity and one is an *in vivo* comet study. The latter study (Jalil et al 2017) is an abstract and no full report is available. Consequently, the validity of the studies cannot be assessed. Therefore, the information provided by the third party in itself would not be sufficient to adapt the standard information requirement.

c) Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the modified study with the two nanoforms described in your dossier and characterized in the reports you submitted:

In vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route on the following tissue: bone marrow or peripheral blood combined with an

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

It is at your discretion to perform 1) the micronucleus analysis both in bone marrow and in blood, and 2) the comet assay also in blood and to carry out the proposed additional toxicokinetic study during the testing program.

d) *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 negative test result can be considered reliable 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.

Please ensure that the combination of the micronucleus and comet assay studies will not impair the validity of and the results from each individual study. Careful consideration should be given to the tissue sampling for the comet assay and for the micronucleus test. (see OECD TG 489, e.g. Bowen et al. 2011[2]).

You are 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".

[2] 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

You may consider examining gonadal cells in the comet assay, 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.

Appendix 2: Procedural history

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 6 June 2017.

ECHA held a third party consultation for the testing proposals from 28 February 2018 until 16 April 2018. ECHA received information from third parties (see Appendix 1).

This decision does not take into account any updates after **11 March 2019**, 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 proposals for amendment.

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-65 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 requests in this decision will result in a notification to the enforcement authorities of the Member States.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests 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 by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally the composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values and other parameters relevant for the property to be tested. In this case the reporting shall also include analysis of size, shape, crystallinity, surface treatment and functionalisation and specific surface area of the tested nanoform. Without such detailed reporting, ECHA will not be able to confirm that the test material is relevant for the substance and to all the registrants of the substance.