Decision number: TPE-D-2114311173-66-01/F Helsinki, 26 November 2015

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

For Flue dust, portland cement, CAS No 68475-76-3 (EC No 270-659-9), 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 proposals submitted as part of the registration dossier in accordance with Articles 10(a)(ix) and 12(1)(e) thereof for Flue dust, portland cement, CAS No 68475-76-3 (EC No 270-659-9), submitted by [redacted] (Registrant).

- *In vivo* gene mutation on lung cells,
- Sub-chronic toxicity study (90 days), (OECD Guideline 413) inhalation route

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 1000 tonnes or more per year. This decision does not take into account any updates submitted after the deadline for updating (14 March 2015) communicated to the Registrant by ECHA on 05 February 2015.

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.

ECHA received the registration dossier containing the above-mentioned testing proposals for further examination pursuant to Article 40(1) on 20 September 2010.

ECHA held a third party consultation for the testing proposals from 16 August 2011 until 30 September 2011. ECHA did not receive information from third parties.

On 12 November 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.

By 20 December 2014 the Registrant did not provide any comments on the draft decision to ECHA.

On 23 July 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 were submitted.

On 28 August 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 amended the draft decision.

On 7 September 2015 ECHA referred the draft decision to the Member State Committee.

By 28 September 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 13 October 2015 in a written procedure launched on 1 October 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 additional test pursuant to Article 40(3)(c) 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 IX, Section 8.4., column 2; test method: OECD 489) in rats, via inhalation route, on the following tissues: liver and lungs.

while the originally proposed test for an *in vivo* gene mutation test on lung cells proposed to be carried out using the registered substance is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

The Registrant shall carry out the following modified test pursuant to Article 40(3)(b) of the REACH Regulation using the indicated test method and the registered substance subject to the present decision:

2. Sub-chronic toxicity study (90-day) in rats, inhalation route (Annex IX, Section 8.6.2.; test method: OECD 413). The test shall be performed using nose-only exposure and shall include bronchoalveolar lavage (BAL) analysis.

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 by **2 June 2017** 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 proposals submitted by the Registrant for the registered substance.

A. Tests required pursuant to Article 40(3)

1. *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 an *in vitro* micronucleus study performed according to the test method OECD 487 with the registered substance that shows positive results. The positive results indicate that the substance is inducing chromosomal aberrations under the conditions of the test.

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 the Registrant was correct in considering it necessary to generate information for this endpoint.

The Registrant has submitted a testing proposal for an "*in vivo* gene mutation study in lung cells". The Registrant did not further identify a specific test guideline intended to be used to perform the proposed study.

It is ECHA's understanding from the above testing proposal that the Registrant proposes to perform an *in vivo* study investigating gene mutation. Since positive results were obtained in an *in vitro* micronucleus study performed with the registered substance, indicating that this substance induces chromosome aberrations under the conditions of this test, ECHA considers that the necessary *in vivo* follow-up study should have the potential to investigate chromosome aberrations. In the absence of an unambiguous reference to a specific test method, and without a detailed description of the experimental protocol proposed to be carried out, ECHA considers that the Registrant has not demonstrated that the proposed test is suitable to further investigate the positive results obtained in the *in vitro* micronucleus study.

The Registrant did not provide a justification for the proposal to investigate the genotoxicity of this substance in lung cells. Based on the dusty nature of the registered substance and on the exposure patterns described in the registration dossier, the inhalation route indeed appears to be the most likely route of human exposure.

It is noted that the ECHA Guidance for information requirements and chemical safety assessment R.7a section R.7.7.6.3 indicates that the assessment of the genotoxicity of the substances for which no indications of systemic availability exist can focus on tissues at the site of contact with the organism. No study specifically addressing absorption and distribution of the registered substance is reported in the dossier. The Registrant concludes in section 7.1 of the IUCLID dossier, on the basis of existing toxicological data, that "*after oral exposure some components of the test substance are absorbed to some extent*". However, despite the potential systemic availability of some constituents of the substance subject to this decision after oral exposure, ECHA is of the opinion that the dusty nature of the substance, the exposure pattern reported in the dossier and the local effects observed in an inhalation acute toxicity study justify investigations on the potential of this substance to cause chromosomal aberrations in pulmonary tissues after inhalation exposure. Therefore, in light of the physicochemical properties of the substance (dust), ECHA considers that testing by the inhalation route is appropriate.

ECHA considers that the Comet assay is an appropriate test to further investigate effects on chromosomal aberrations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment, chapter R.7.7.1. and figure R.7.7-1 (August 2013). This test method presents the advantage of being able to detect these genetic defects in any tissues of the exposed animal, making it a method of choice for investigations in site of contact tissues including pulmonary tissues.

The Registrant is requested to examine tissues sampled from the lungs and from the liver in the Comet assay. The lung was chosen due to inhalation administration to evaluate mutations at the site of contact with the body. The liver was chosen to evaluate mutationsin the organ that is primarily responsible for metabolism of xenobiotics.

Therefore, pursuant to Article 40(3)(c) 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, via inhalation route, on the following tissues: liver and lungs whereas the testing proposal for an "*in vivo* gene mutation study in lung cells" made by the Registrant is rejected pursuant to Article 40(3)(d) of the REACH Regulation.

Notes for consideration by the Registrant

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 examining gonadal cells when conducting the requested comet assay, as it would optimise the use of animals. ECHA notes that positive results in whole gonads are not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive results would indicate that the tested substance(s) and/or its metabolites 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.

The Registrant is reminded that the comet assay test guideline OECD 489 gives the possibility for it to be integrated with the requested 90 day repeated dose toxicty study (OECD 408 or 413) (see point 7 of the guideline). In order to ensure that the generated data will be acceptable to cover the data requirement for the *in vivo* mammalian alkaline comet assay the issues referred to below need to be considered by the Registrant in case he decides to combine the two studies:

- the maximum tolerated dose (MTD) in the sub-chronic toxicity study may be lower than the MTD in a standard (2-day) comet assay. Negative results for a comet assay obtained in a combined sub-chronic /comet assay study where the maximum tolerated dose is significantly lower than would be expected in a comet assay performed as stand-alone study may lead to an underestimation of the potential of the test substance to cause genotoxicity and thus be considered inadequate to fulfill the information requirement of Annex IX, Section 8.4., column 2.
- The age of the animals and the corresponding historical controls: the laboratory performing the study should have historical control data for the comet assay for animals at the end of the 90-day chronic toxicity study (*i.e.* 13 weeks older than in the comet assay).
- An additional group of animals, *i.e.* positive control group, should be added to the sub chronic toxicity study protocol to demonstrate that the induced response are compatible with those generated in the historical positive control database.
- Careful consideration should be given to the logistics involved in tissue sampling for comet analysis alongside the requirements of tissue sampling for other types of toxicological assessments. Harvest 24 hours after the last dose, which is typical of a general toxicity study, is not appropriate for the comet assay where samples are usually collected 2-6 hours after the last treatment (see OECD 489, paragraph 33).

2. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2)

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test but modifying the conditions under which the test is to be carried out.

A sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The Registrant has submitted a testing proposal for a sub-chronic toxicity study (90 day) the inhalation route (OECD 413).

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.6.2. of the REACH Regulation.

The Registrant proposed testing by the inhalation route. In light of the physico-chemical properties of the substance, an insoluble dust of inhalable size classified as irritating to the skin and damaging the eyes, and the information provided on the uses and human exposure *i.e.* uses with spray application, roller application or brushing, production of preparations or articles by tabletting, compression, extrusion, pelletisation, ECHA considers that testing by the inhalation route is most appropriate.

ECHA notes that the substance is an insoluble dust of inhalable size with a respirable fraction of about 10% according to the non-confidential registration. Hence the alveoli may be the primary site of retention due to the low clearance rate of insoluble particles from the alveoli. BAL was not conducted in the sub-acute inhalation toxicity study and hence no information is available for influx of inflammatory cells and pro-inflammatory cytokines/chemokines and other sensitive markers of inflammation for this substance. Due to the high potential for long-term human exposure by inhalation for this substance a more sensitive test is warranted. Read across from Portland cement clinker and different common cement types showed that all cement dusts induced a significant TNF-release in a macrophage cell line (NR8383) indicative of macrophage activation and inflammogenic potential. Therefore, in order to investigate the potential particle-induced or irritant inflammation in the lower lung, especially long term and dose-dependent effects due to the high retention of insoluble, respirable particles ECHA is requesting that BAL is being performed in the inhalation 90-day (sub-chronic) study. BAL fluid shall be analyzed for total and differential cell count, protein content, lactate dehydrogenase and pro-inflammatory cytokines/chemokines such as TNF-alpha. Other parameters should be considered by the Registrant taking into account potential effects of the substance in the lung. The Registrant should further consider that the preferred mode of exposure is nose-only and that particulate materials should be subjected to mechanical processes. To allow for exposure of all relevant regions of the respiratory tract, aerosols with mass median aerodynamic diameters (MMAD) ranging from 1 to 3 µm with a geometric standard deviation (σg) in the range of 1.5 to 3.0 are recommended as specified in OECD 413 test guideline

The Registrant did not specify the species to be used for testing. According to the test method OECD 413 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

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: Sub-chronic toxicity study (90-day) in rats, inhalation route (test method: OECD 413). The test shall be performed using nose-only exposure and shall include bronchoalveolar lavage (BAL) analysis.

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 studies 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.

In relation to the proposed tests, the sample of substance used for the new studies must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is within the specifications of the substance composition that are given by the joint registrants. It is the responsibility of all joint registrants of the same substance to agree to the tests proposed (as applicable to their tonnage level) and to document the necessary information on their substance composition.

In addition, it is important to ensure that the particular sample of substance tested in the new studies 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 studies 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 studies 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 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[1] by Guilhem de Seze, 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.