EUROPEAN CHEMICALS AGENCY

Decision number: CCH-D-2114312646-52-01/F Helsinki, 30 March 2016

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

For barium dodecairon nonadecaoxide, EC No 234-974-5 (CAS No 12047-11-9), 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 41(1) of the REACH Regulation ECHA has performed a compliance check of the registration for barium dodecairon nonadecaoxide, EC No 234-974-5 (CAS No 12047-11-9), 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. ECHA stresses that it has not checked the information provided by the Registrant and other joint registrants for compliance with requirements regarding the identification of the substance (Section 2 of Annex VI).

This decision is based on the registration 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 27 February 2015, i.e. the date when the draft decision was notified to the Registrant under Article 50(1) of the REACH Regulation.

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 4 November 2014.

On 27 February 2015 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 7 April 2015 ECHA received comments from the Registrant on the draft decision.

The ECHA Secretariat considered the Registrant's comments. On basis of the Registrant's comments, Sections II and III were amended.

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

On 28 August 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.

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 proposal for amendment. The Member State Committee took the comments on the proposal for amendment of the Registrant 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. Information required

Pursuant to Articles 41(1), 41(3), 10(a)(vi) and/or (vii), 12(1)(e), 13 and Annexe 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 alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD 489), in rats, inhalation route or using tracheal instillation as justified, target tissue lung.

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.

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 **6 April 2018** 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)(e) of the REACH Regulation, a technical dossier for a substance manufactured or imported by the Registrant in quantities of 1000 tonnes or more per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation.

"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." Hence, 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 Cell Micronucleus Test performed according to OECD 487 with the registered substance that shows mutagenic activity at the highest dose levels tested. The Registrant reports and recognises these results as positive and states that "the test substance shows a statistically significative increment in micronucleate cells. This increment could indicate an induction of chromosome breaks and/or gain or loss in cultured mammalian cells".

However, the Registrant did not provide an appropriate *in vivo* somatic cell test to detect any chromosomal aberrations.

Instead, the Registrant has sought to adapt the information requirement stating that "the uncertainties, which are arisen from the *in vitro* test results, would make desirable a verification by means of an *in vivo* test, as it is foreseen by the Regulation EC 1907/2006. However, toxicokinetics evidence do not support the adsorption (after oral administration) and the subsequently reaching of the target tissue by the substance, making non reliable the validated *in vivo* mutagenicity tests". The Registrant furthermore refers to B.11 (*In Vivo* Mammalian Bone Marrow Chromosome Aberration Test) and B.12 (*In Vivo* Mammalian Erythrocyte Micronucleus Test) sections of the Regulation CE 440/2008 testing guidelines stating that "If there is evidence that the test substance, or a reactive metabolite, will not reach the target tissue, it is not appropriate to use this test".

ECHA agrees that the abovementioned tests are not appropriate in the case of the registered substance because of the potentially insufficient systemic availability of the registered substance after oral administration. However, the argument is not convincing to justify waiving the information requirement, because it is possible to fulfil the information requirement by other test methods, such as the comet assay (OECD 489). The Registrant recognises the potential for lung toxicity following inhalation exposure by classifying the registered substance for Specific Target Organ Toxicity (STOT) Single/Repeated Exposure 2, affected organ lung. In the absence of an acceptable adaptation argument for waiving the information requirement and taking into account the above considerations, ECHA considers it necessary to perform an *in vivo* assay addressing chromosomal aberrations examining site of contact tissues as described in the ECHA *Guidance* on information requirements and chemical safety assessment (Version 3.0, August 2014), Chapter R.7a, Section R.7.7.1. and Figure R.7.7-1.

ECHA concludes that the Comet assay (OECD 489 guideline, adopted on 26 September 2014) is an adequate test method to conduct such examination. ECHA considers the lung as the appropriate target tissue following inhalation exposure to investigate potential for chromosome aberrations at a direct site of contact.

In its official comments to the draft decision the Registrant proposed to consider, as its first-line option, to fulfil the information requirement of Annex IX 8.4. mutagenicity using a read-across approach and iron oxides data as a worst case scenario. As a second-line option, the Registrant proposed to perform a feasibility study for use of intra-tracheal administration and consequently, taking account of the outcome of the feasibility study, the Comet assay. The Registrant also wishes to prolong the deadline for updating the dossier accordingly to 24 months to allow time to conduct the preliminary feasibility study before the Comet assay.

According to the Registrant's comments, "considering all available information for hexaferrites, iron derivatives and barium derivatives could properly satisfy the information requirements in order to better characterize the mutagenicity activity of barium hexaferrite." ECHA recognises the read-across adaptation possibility but notes that the criteria for the adequate and reliable documentation of the read-across approach required in Annex XI, Section 1.5 are not met at this stage. The Registrant did not provide robust study summaries of the genotoxicity studies addressing the genotoxic potential of the referred compounds and only a preliminary evaluation of possible read-across strategy has been performed. Therefore, ECHA concludes that in the absence of both an an adequate read-across justification and the supporting data the read-across cannot be fully evaluated or accepted.

ECHA has made the following observations regarding the Registrant's read-across argumentation:

(i) The Registrant states that several substances might be suitable to use as sources of data for read-across, e.g. strontium hexaferrite and iron derivatives (e.g. iron oxide) and barium derivatives. Based on the preliminary results, the Registrant states that "*these substances*" have very low water solubility, absorption via oral route is poor and acute oral toxicity is low (LD50 > 2000 mg/kg), and therefore, they can be used to predict the mutagenicity for barium hexaferrite.

ECHA notes that, except for strontium hexaferrite, the Registrant has not specified which substances have been used for this analysis. Therefore, it is unclear which substances are proposed to be used as analogue substances.

The Registrant has concluded that since the substances have similar water solubility and oral absorption and show low acute oral toxicity, they can be used for the read-across approach to address the datagap for mutagenicity i.e. a comet assay. ECHA notes that the Registrant has not assessed the structural similarities between the substances and has not explained how the different structures of the substances proposed to be used for read-across may impact the toxicological properties, and in particular, for mutagenicity.

(ii) The Registrant states that strontium hexaferrite does not show genotoxicity in Ames test, *in vitro* mammalian cell gene mutation and *in vitro* mammalian chromosome aberration test.

ECHA notes that only results (negative) of the genotoxicity studies conducted with strontium hexaferrite were mentioned, without providing the respective robust study summaries, and therefore the reliability and adequacy of these studies cannot be evaluated.

(iii) The Registrant states that no genotoxicity "*alerts*" were found for barium compounds in the literature.

ECHA notes that the Registrant has not specified which barium compounds were studied.

(iv) The Registrant states that for some iron derivatives, some "*positive evidence exists on the mutagenicity in in vitro models*", which were not confirmed in the *in vivo* studies. He further summarises results of two *in vivo* studies conducted with Fe_2O_3, B(a)P and B(a)P coated onto Fe_2O_3 particles.

ECHA notes that the study reports from the *in vitro* studies were not provided in the registration dossier. ECHA further notes that in the *in vivo* study by Garcon *et al* (2001), the effects on lipid peroxidation in the lungs of Fe_2O_3, B(a)P and B(a)P coated onto Fe_2O_3 particles was investigated and therefore, the study is not considered adequate to fulfil the information requirement for mutagenicity (Annex IX, Section 8.4., column 2, Comet assay). The same substances were investigated in a non-guideline Comet assay (Garry *et al*, 2003). ECHA notes that the study design seems to deviate from the OECD test guideline 489. There are apparently conflicting statements regarding the effects of Fe_2O_3 ("*No damage was observed in cell from the four investigated organs in rats treated with iron oxide alone*" and "*It is generally assumed that iron play a critical role in the Haber-Weiss reaction, which would produce free radicals and reactive oxygen species (ROS), both able to subsequently induce DNA strand breaks*". Moreover, as stated in section (i) above, the structural similarity of the registered and analogue substances, e.g. Fe_2O_3, has not been established. In the absence of a robust study summary for this study in the registration dossier, ECHA could not investigate the impact of such deviations on the relevance and adequacy of the results obtained in this study for risk assessment and C&L purposes.

(v) The Registrant concludes that information from hexaferrites, iron and barium derivatives could be used for a read-across approach and iron oxides could represent the worst case scenario.

ECHA notes that, notwithstanding the registrant's preliminary evaluation of the read-across and brief summaries of information provided, the requirements of Annex XI, Section 1.5. are not met as adequate and reliable documentation has not been provided. In particular, the identity of the proposed analogue substances to be used for the read-across approach is unclear, no robust study summaries of the studies referred to in the comments have been provided, the structural similarities and impact(s) due to differences in the structures of possible analogue substances on toxicity and the basis for selecting which analogous substances represent the worst-case have not been adequately addressed. Therefore, ECHA is not able to assess the Registrant's claim of iron oxides representing the worst case scenario.

Based on the deficiencies listed above, ECHA considers that the current read-across approach is not acceptable.

Furthermore, in its comments to the draft decision, the Registrant raises a concern that administration via inhalation would lead to unreliable/unpredictable exposure levels compromising the overall reliability of the study and suggests that intra-tracheal instillation could be a more suitable method of administration. ECHA notes that, if the Registrant considers the inhalation route as not appropriate and decides to perform a Comet assay via intra-tracheal administration instead, a scientifically sound justification is required which would also explain why this method of administration would ensure adequate exposure of the target tissue (lung). It is noted that according to OECD 489, inhalation or intra-tracheal route may be chosen. It is at the discretion of the Registrant if a preliminary study examining the feasibility of Comet assay via instillation is needed and should be performed.

Furthermore, the Registrant expressed a concern whether the results of the study would be reliable as there is a possibility of false positive results that could be obtained with Comet assay due to cytotoxic properties of the registered substance. The Registrant reports that some evidence of cytotoxicity has been observed cells exposed *in vitro* to the registered substance. ECHA acknowledges that cytotoxicity that may be observed under the *in vivo* conditions of the of the Comet assay is an issue to be considered when conducting the assay and interpreting the results. According to OECD 489, "where positive or equivocal findings are observed solely in the presence of clear evidence of cytotoxicity, the study would be concluded as equivocal for genotoxicity unless there is enough information that is supportive of a definitive conclusion." ECHA further notes that whilst, according to OECD 489, "no definitive list of histopathological changes that are always associated with increased DNA migration is available" it is also stated that "many measures of cytotoxicity have been proposed and of these histopathological changes are considered a relevant measure of tissue toxicity". Therefore, ECHA considers that the Comet Assay guideline sets out how measurement of cytotoxicity might be achieved and how increases in DNA migration in the presence of clear evidence of cytotoxicity can be interpreted. Moreover, ECHA considers that speculating on cytotoxicity at this stage is inconclusive as it is not demonstrated that the cytotoxicity observed in the *in vitro* study by Kim *et al* 2005 is relevant for the *in vivo* study requested in the draft decision.

The Registrant clarifies in its official comments to the draft decision that consumers and general public are not expected to be exposed to the registered substance via inhalation route as it is only available incorporated in articles. However, ECHA notes that based on the information made available in the registration dossier and in the Registrant's comments to the draft decision that, as barium hexaferrite is manufactured as dust and it is usually available as such in the supply chain for industrial and professional uses until it is incorporated in mixtures and articles, potential for human exposure exists.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, the Registrant is required to submit the following information derived with the registered substance subject to the present decision:

In vivo mammalian alkaline comet assay (Annex IX, Section 8.4., column 2; test method: OECD 489), in rats, inhalation route or using tracheal instillation as justified, target tissue lung.

Note 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.

IV. Adequate identification of the composition of the tested material

ECHA stresses that the information submitted by the Registrant and other joint registrants for identifying the substance has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation . 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.