EUROPEAN CHEMICALS AGENCY

Helsinki, 01 September 2015

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DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For diallyl phthalate, CAS No 131-17-9 (EC No 205-016-3)

Addressee: Registrant(s)[1] of diallyl phthalate (Registrant(s))

This decision is addressed to all Registrant(s) of the above substance with active registrations on the date on which the draft for the decision was first sent for comment with the exception of the cases listed in the following paragraph. A list of all the relevant registration numbers subject to this decision is provided as an annex to this decision.

Registrants holding active registrations on the day the draft decision was sent are *not* addressees of this decision if they are: i) Registrant(s) who had on that day registered the above substance exclusively as an on-site isolated intermediate under strictly controlled conditions and ii) Registrant(s) who have ceased manufacture/import of the above substance in accordance with Article 50(3) of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation) before the decision is adopted by ECHA.

Based on an evaluation by the Ministry of Health, Social Services and Equality as the Competent Authority of Spain (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 15 January 2015, i.e. the day on which the draft decision was notified to the Registrant(s) pursuant to Article 50(1) of the REACH Regulation.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Spain has initiated substance evaluation for diallyl phthalate, CAS No 131-17-9 (EC No 205-016-3) based on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to human health/CMR, particularly mutagenicity, exposure/wide

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

dispersive use and consumer use, diallyl phthalate was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013. The updated CoRAP was published on the ECHA website on 20 March 2013. The Competent Authority of Spain was appointed to carry out the evaluation.

The evaluating MSCA considered that further information was required to clarify the above mentioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 19 March 2014.

On 29 April 2014 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant commenting phase

By 5 June 2014 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay.

The evaluating MSCA considered the comments received from the Registrant(s). On basis of this information, Section II was amended. The Statement of Reasons (Section III) was changed accordingly.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 15 January 2015 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, two Competent Authorities of the Member States submitted proposals for amendment to the draft decision.

On 20 February 2015 ECHA notified the Registant(s) of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment received and did not amend the draft decision.

Referral to Member State Committee

On 2 March 2015 ECHA referred the draft decision to the Member State Committee.

By 23 March 2015 the Registrant(s)' comments were provided on the proposed amendments. The evaluating Member State Committee took these comments into account.

After discussion in the Member State Committee meeting on 20 to 23 of April 2015, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 21 April 2015. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods (in accordance with Article 13(3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

1. Transgenic Rodent Somatic and Germ Cell Mutation Assays (test method: EU B.58/OECD TG 488). The test shall be conducted in mice or rats treated for 28 days, via oral route, and tissues (stomach, liver and bone marrow) shall be harvested three days after the cessation of the treatment. Mutation frequency shall be assessed in stomach, liver and bone marrow. The germ cells shall be sampled and stored for analysis if positive results are obtained in any of the somatic cells.

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall also submit the following information regarding the registered substance subject to the present decision:

2. Worker exposure assessment:
   a) Conduct a higher tier (Tier 2) exposure assessment, in accordance with ECHA Guidance on information requirements and chemical safety assessment. Chapter R.14: Occupational exposure estimation (ECHA, 2012), for dermal exposure to workers in exposure scenarios 1, 2 and 3.
   b) Provide further information on personal protective equipment (e.g. gloves) regarding the type of material to be used and the breakthrough times for the gloves.

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **08 March 2017**[2] an update of the registration(s) containing the information required by this decision for points 1 and 2 of this Section II, including robust study summaries and, where relevant, an update of the Chemical Safety Report.

III. Statement of reasons

1. TGR assay (EU B.58/OECD TG 488) in mice or rats by oral route

Initial grounds for concern related to suspected mutagenic potential were confirmed by the assessment of the available information. On the basis of the IUCLID data, no clear conclusions about the mutagenic potential of diallyl phthalate (DAP) can be derived.

The genotoxic potential of DAP has been assessed in *in vitro* and *in vivo* assays. The Registrant(s) provided results for several bacterial reverse mutation assays (EU B.13/14, OECD TG 471). A weakly positive result was only obtained in *Salmonella typhimurium* strain TA1535, in the absence of metabolic activation ([redacted], 1986). In addition, a weak positive response was also observed in *E. coli* WP2 with metabolic activation (OECD TG 472) (MOL, 2000, quoted in OECD SIDS, 2004). Other bacterial mutagenicity tests have been reported as negative with and without exogenous metabolic activation ([redacted], 1977; Seed *et al.*, 1982; Zeiger *et al.*, 1985, quoted in OECD SIDS, 2004; Sato *et al.*, 1994). On the contrary, clear positive responses were observed in mammalian cells, in the presence of exogenous metabolic activation: DAP induced chromosomal aberrations (OECD TG 473) and sister chromatid exchanges in Chinese hamster ovary cells (OECD TG 479) (Gulati *et al.*, 1989). Micronucleus formation was also found in an *in vitro* micronucleus test (MOL, 2002, quoted in OECD SIDS, 2004). Furthermore, the Registrant(s)

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

have submitted a positive *in vitro* mammalian cell gene mutation test (OECD TG 476) (Myhr and Caspary, 1991). The positive result was clearer in the presence of metabolic activation. With respect to colony size distribution, both large and small colonies were induced, but predominantly the small ones, which is an indication of clastogenicity.

In relation to the *in vivo* testing data on mutagenicity, the Registrant(s) have provided negative results for an *in vivo* mouse micronucleus test (OECD TG 474) (Shelby *et al.*, 1993) and equivocal results for an *in vivo* chromosome aberration test in mouse bone marrow cells (Shelby and Witt, 1995). Both studies were only available as published in scientific journals. In the chromosome aberration test, there was a small but statistically significant increase in the number of chromosome aberrations at the high dose, only in one of two trials conducted under the same conditions. The dose-effect relationship was not consistent. The biological significance of this result is not clear. Based on the available information, the substance was originally self-classified by the Registrant(s) as Muta. 2. Even though the classification for cell germ mutagenicity was omitted by mistake in Section 2 of the updated IUCLID dossier of December 2013, a new update of the IUCLID dossier from June 2014 included again the original one.

The potential of DAP to cause gene mutations has been investigated only *in vitro*. The positive results in two bacterial reverse mutation assays and in one mammalian cell gene mutation test show an alert also for gene mutation that has not been investigated *in vivo*.

Clarification is needed on the potential of this substance to be mutagenic in somatic and germ cells and, if positive results were obtained, on its mechanism of action. Therefore, an appropriate guideline and well conducted *in vivo* assay is necessary to clarify the genotoxic potential observed in some *in vitro* studies. It would be appropriate to investigate the potential to cause gene mutation *in vivo*. An *in vivo* transgenic rodent gene mutation assay is able to detect stable mutations and has the advantage that it permits to investigate gene mutation in both somatic and germ cells and its results would be adequate for classification and labelling purposes.

Registrant(s) are requested to perform the TGR assay in stomach, liver and bone marrow. The reasons for tissues selection, as outlined in the test guideline (OECD TG 488 paragraphs 37 and 38), are that the stomach was chosen due to oral administration and to evaluate mutation at the initial site of contact. This is also consistent with the ability of the substance to act as a sensitizer (the substance is self-classified as Skin sens. 1B). In addition, it is a rapidly dividing tissue. Liver was chosen as the primary target organ of DAP that is also one slowly dividing tissue. Finally, the bone marrow was selected because it is a rapidly dividing cell population distant of the initial site of contact. The Registrant(s) shall collect and store male germ cells for potential further analysis of germ cell mutagenicity in case positive results are obtained from the somatic cells.

In their comments to the draft decision, the Registrant(s) acknowledge the request for an OECD TG 488. Nevertheless, the Registrant(s) question that the substance or its relevant metabolites reach the selected tissues. They state not to be aware of any studies concerning metabolism of DAP by the oral route and propose carrying out a 7 day range finding study with toxicokinetic endpoints to determine systemic exposure to DAP and the relevant metabolites. This would help assess the rationale for determining what the exposure of cells in the stomach, liver and bone marrow will be.

Although the rate of metabolism via other routes different from the *iv* has not been investigated, Eigenberg *et al.* (1986) show that the same metabolites were formed after *iv* and oral administration of DAP to rats and mice. The available data on toxicokinetic and the repeated dose toxicity studies are enough to demonstrate that the substance or its main metabolites reach the relevant tissues. Therefore, it is not considered necessary to perform

the proposed study.

Initially, a mammalian erythrocyte micronucleus test had been required as a second *in vivo* assay in case of a negative result in the TGR study. The aim was to remove the uncertainty created by the results of the *in vivo* chromosome aberration tests.

The Registrant(s) noted the request for an OECD 474 but were particularly concerned about the additional animal testing. They underline the validity of the available *in vivo* studies despite the deficiencies in the reporting and, consequently, classify the substance in accordance with the results.

Taking into account that in the last updated registration dossier the substance is actually self-classified by the Registrant(s) as Muta. 2, it is considered now that the Registrant(s) has sufficiently addressed the potential concern, if any, using a worst case approach. Then, an additional study for chromosome aberration effect is considered no longer necessary in the scope of substance evaluation. Therefore, based on this and on animal welfare issues, the information request for an *in vivo* micronucleus test under section II was removed.

During the consultation with MSCAs, a proposal for amendment (PfA) was submitted by a Member State to replace the requested test, OECD TG 488–TGR, with a mammalian cell spermatogonial chromosomal aberration test (OECD TG 483) in order to assess the germ cell clastogenicity/aneugenicity of the susbstance.

In response to this PfA, the Registrant(s) indicated again their agreement with the required test OECD TG 488. Additionally, they suggested performing a micronucleus assessment on peripheral blood samples taken from animals exposed to DAP over the 28-day dosing regimen of the OECD TG 488 study. This would address the concern related with clastogenicity/aneugenicity raised by the MSCA in their PfA.

This proposal could clarify the uncertainty concerning the chromosome aberration effect resolved by the Registrant(s) with the self classification. However, it is noted that there is no validated protocol available for the combination of both tests. Although this combination possibility is not foreseen in any of the individual OECD guidelines, i.e. OECD TG 488 or OECD TG 474, paragraph 37.c of the OECD TG 474 adopted on 26 September 2014 gives the possibility to integrate the micronucleus test with repeated-dose toxicity studies. The TGR assay is based on a repeated-dose regimen with daily treatments for a period of 28 days. In addition, there are publications reporting the combination of TGR experiments with the peripheral blood micronucleus assay (OECD, 2009).

Therefore, it is left at the discretion of the Registrant(s) to perform the additional micronucleus assessment on peripheral blood provided that all the conditions established by both individual studies are met and well documented.

Taking into account the above considerations, the Registrant(s)' comments on the proposals for amendment did not lead to an amendment of the information requirement.

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following studies using the registered substance subject to this decision:

Transgenic Rodent Somatic and Germ Cell Mutation Assay (test method: EU B.58/OECD TG 488). The test shall be conducted in mice or rats treated for 28 days via oral route and tissues (stomach, liver and bone marrow) shall be harvested three days after the cessation of the treatment. Mutation frequency shall be assessed in stomach, liver and bone marrow. The germ cells shall be sampled and stored for analysis if positive results are obtained in any of the somatic cells.

Note for consideration by the Registrant(s)

Once the results of this study are available, the evaluating MSCA will consider the need to request further information in order to assess any remaining concern for mutagenicity.

2. Worker Exposure Assessment

Initial grounds for concern relating to exposure in particular to workers were confirmed by the assessment of the available information.

The information requested is required to evaluate the risk to human health arising from occupational exposure to DAP.

a) Conduct a higher tier (Tier 2) exposure assessment for dermal exposure to workers in exposure scenarios 1, 2 and 3

The Registrant(s) have conducted a human exposure assessment as requested in Article 14 of the REACH Regulation. Occupational exposure assessment was based on tier 1 tool ECETOC TRA version 3. According to this assessment, dermal exposure may contribute significantly to overall exposure due to the very low vapour pressure of this substance (0.02 Pa at 25 °C).

The Registrant(s) have not been able to prove that dermal exposure to DAP is adequately controlled in scenarios 1 and 3 (PROC 19 and PROC 10). The Registrant(s) have stated in the CSR that conducting site monitoring is necessary for these tasks.

In their assessment, the Registrant(s) have considered LEV effective for dermal exposure reduction for some activities in scenarios 1, 2 and 3. In this way, ECETOC TRA may underestimate dermal exposure levels. In addition, an effectiveness of 95% for protective gloves is considered by the Registrant(s) in all scenarios. However, specific information regarding the type of glove material, its thickness and breakthrough time is lacking. According to these choices, exposure in scenario 2 may have also been underestimated and risk may not be adequately controlled.

Therefore, the Registrant(s) are required to perform a higher tier (Tier 2) dermal exposure assessment, in accordance with ECHA Guidance on information requirements and chemical safety assessment, Chapter R.14: Occupational exposure estimation (ECHA, 2012), for dermal exposure to workers in exposure scenarios 1, 2 and 3. Where a higher tier model is used, a justification of the model chosen, a description of the input parameters required for modelling and assumptions done should be included. Representative and reliable measured exposure data could also be provided. In this case, data should be collected and analysed according to internationally recognised guidelines.

b) Provide further information on personal protective equipment regarding the type of material to be used and the breakthrough times for the gloves

The Registrant(s) have estimated workplace dermal exposure to DAP using the tier 1 tool ECETOC TRA version 3. The assessment has taken into account the effect of PPE on reducing exposure. Gloves are described with a reference to EN 374 standard and reported to be used with specific activity training. Breakthrough time is only mentioned. An effectiveness of 95% for protective gloves is considered in the CSR for all exposure scenarios. However, specific information regarding the type of glove material, its thickness and breakthrough time is lacking. This information is necessary to assess the protection efficacy assigned to gloves in the CSR.

Without this information, it cannot be demonstrated that risk management measures described in the exposure scenarios ensure a safe use of the substance.

The Registrant(s) are accordingly required to provide detailed specifications of the personal protection equipment. In particular for gloves, this includes information on the type of material and its thickness and the typical or minimum breakthrough time of the glove material.

IV. Adequate identification of the composition of the tested material

In relation to the required experimental studies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the tests subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the tests must be shared by the Registrant(s).

V. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 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 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[3] by Leena Ylä-Mononen, Director of Evaluation

Annex 1: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

[3] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Annex II: References

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