Helsinki, 30 March 2016

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

For 1,2-dichlorobenzene, CAS No 95-50-1 (EC No 202-425-9)

Addressees: Registrant(s)[1] of 1,2-dichlorobenzene (Registrant(s))

This decision is addressed to the Registrant(s) of the above substance with active registration pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. Where Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided as an Annex to this decision.

Based on an evaluation by the Hungarian National Institute of Chemical Safety as the Competent Authority of Hungary (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 the REACH Regulation.

This decision is based on data provided in the registration dossier(s) on 1 August 2014.

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 Hungary initiated substance evaluation for 1,2-dichlorobenzene CAS No 95-50-1 (EC No 202-425-9) based on registration dossier(s) submitted by the Registrant(s) 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/suspected CMR properties, suspected repeated dose toxicity, possible exposure, as well as wide dispersive use and high aggregated tonnage, 1,2-dichlorobenzene was included in the Community rolling action plan (CoRAP) for substance evaluation pursuant to Article 44(2) of the REACH Regulation to be evaluated in 2013. The CoRAP was published on the ECHA website on 20 March

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

2013. The Competent Authority of Hungary was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding potential reproductive toxicity.

The evaluating MSCA considered that further information was required to clarify the concerns on repeated dose toxicity and mutagenic properties, as well as an additional concern on potential reproductive toxicity raised during the substance evaluation. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information and submitted the draft decision to ECHA on 20 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(s)' commenting phase

By 5 June 2014 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay. By 1 August 2014 the Registrant(s) submitted an update of the registration dossier. The evaluating MSCA considered the comments received from the Registrant(s) and the dossier update, but did not modify the draft decision.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 3 September 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, four Competent Authorities of the Member States and ECHA submitted proposals for amendment (PfAs) to the draft decision. On 5 October 2015, ECHA referred the draft decision to the Member State Committee.

On 9 October 2015 ECHA notified the Registrant(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 amended the draft decision by revising the test requirements on mutagenicity and proposing to address reproductive toxicity in a follow-up evaluation, if necessary.

Referral to Member State Committee

On 19 October 2015 ECHA referred the draft decision to the Member State Committee.

By 9 November 2015, in accordance to Article 51(5), the Registrant(s) provided comments on the proposals for amendment. In particular, the Registrant(s) informed the evaluating MSCA that they had stopped producing the substance for professional use as laboratory reagent. By this, the concerns related to possible exposure and wide

dispersive use are not substantiated anymore. Consequently, the evaluating MSCA further revised the draft decision and removed the information requirements on repeated dose toxicity and reproductive toxicity.

After discussion in the Member State Committee meeting on 7 to 11 December 2015, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 8 December 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/instructions (in accordance with Article 13 (3) and (4) of REACH Regulation) and the registered substance subject to the present decision. The requested tests shall be conducted with the registered substance, 1,2-dichlorobenzene, with the highest possible concentration of the identified impurities as specified in the registration dossiers (EC No 202-425-9). The tests shall be implemented in a tiered approach, as follows:

Tier 1. *In vivo* Mammalian Alkaline COMET Assay (test method: OECD 489) combined with *In vivo* Mammalian Erythrocyte Micronucleus Test (test method: OECD 474) (further referred as COMET Assay and Micronucleus Test, respectively)

The tests shall be conducted on rat and by oral route (gavage). For the COMET Assay, the following tissues shall be analysed: liver, glandular stomach, duodenum/jejunum and bone marrow. For the Micronucleus Test, the bone marrow shall be analysed. Animals shall be dosed with 1,2-dichlorobenzene 48, 24 and 3 hours prior to sacrifice.

Depending on the results of these tests, the following tests shall also be conducted:

Tier 2a. In case the Micronucleus Test is positive the Mammalian Spermatogonial Chromosome Aberration Test (OECD 483), oral route, in rat is requested (further referred as the Chromosome Aberration Test).

Tier 2b. In case the COMET Assay is positive, Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD 488) also oral route, in rat with 70 days exposure is requested (further referred as the TGR).

In this test the liver, glandular stomach, duodenum/jejunum, bone marrow and male germ cells shall be analysed.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, if based upon the results of the mutagenicity studies the Registrant(s) do not need to perform the Chromosome Aberration Test and the TGR, then the Registrant(s) shall submit to ECHA by **9 October 2017** an update of the registration dossier(s) containing the information required by this

decision[2].

In case the performance of the Chromosome Aberration Test is necessary in line with this decision then the update of the registration dossier(s) shall be submitted to ECHA by [exact date – 36 months *from the date of the decision*][2].

In case the performance of the TGR is needed then the update of the registration dossier(s) shall be submitted to ECHA by [exact date – 42 months *from the date of the decision*][2].

III. Statement of reasons

Based on the evaluation of all relevant information submitted on 1,2-dichlorobenzene and other relevant and available information, ECHA concludes that further information is required in order to complete the evaluation and draw clear conclusions on the mutagenic properties of the substance.

Depending on the results of the above requested tests further risk management measures may be substantiated. In particular, if the result of the Chromosome Aberration Test is positive or 1,2-dichlorobenzene causes mutation in male germ cells in the TGR, then classification as Mutagen 1B of 1,2-dichlorobenzene could be substantiated and also other risk management measures may be warranted. In case the substance causes mutation in other tissues than germ cells in TGR test then the appropriate classification of the substance could be Mutagen 2.

Tier 1. *In vivo* Mammalian Alkaline COMET Assay (OECD 489) combined with *In vivo* Mammalian Erythrocyte Micronucleus Test (test method: OECD 474)

Possible mutagenic properties of 1,2-dichlorobenzene were critically analysed based upon data provided by the Registrant, the available relevant data published in scientific periodicals or referred to in the relevant OECD SIDS Report (2001[3]). ECHA came to the following conclusion on mutagenicity.

1,2-dichlorobenzene proved to be not mutagenic in bacterial reverse mutation tests (Masumori et al. 2001[4]; Shimizu et al. 1983[5]), negative in two *in vitro* cytogenetic assays on Chinese hamster ovary (Loveday et al. 1990[6]) and lung cells (Masumori et al. 2001b[8]) and did not induce unscheduled DNA synthesis in primary rat hepatocytes (Williams et al. 1989[7]).

[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).
[3] OECD (2001). SIDS Initial Assessment Report For SIAM 13 (Bern, 6 - 9 November 2001) - 1,2-Dichlorobenzene. UNEP Publications.

[4] Masumori S, Itakura M, Kikuchi M. (2001). Reverse mutation of o-dichlorobenzene on bacteria. Toxicity testing reports of environmental chemicals. 8: 328-332. Testing laboratory: Article in Japanese.

[5] Shimizu M, Yasui Y, Matsumoto N. (1983). Structural specificity of aromatic compounds with special reference to mutagenic activity in Salmonella typhymurium-a series of chloro- or fluoro-nitrobenzene derivatives. Mutat. Res. 116:217-238.

[6] Loveday KS, Anderson BE, Resnik MA, Zeiger E. (1990). Chromosome aberration and sister chromatid exchange test on Chinese hamster ovary cells *in vitro*. V: Results with 46 chemicals. Environmental and Molecular Mutagenesis 16:272-303.

[7] Williams GM, Mori H, McQueen CA (1989). Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. Mutat. Res. 221:263-286.

Two *in vitro* sister chromatid exchange assays using CHO cells (Loveday et al. 1990[7]; Masumori 2001b[8]) and three *in vitro* gene mutation tests on L5178Y cells (Myhr & Caspary 1991[9]; Kim & Ryu 2007[10]; Tennant et al. 1987[11]) gave positive results with metabolic activation. However, an *in vitro* mammalian cell gene mutation assay on Chinese hamster ovary cells resulted in negative findings (Bioassay Systems. Corp. 1984[12]).

1,2-dichlorobenzene gave negative result in *in vivo* cytogenetic assay on rat bone marrow cells (Reustle & Scriber 1979[13]) and did not cause hepatic DNA damage (alkaline elution) (Kitchin et al. 1992[14]).

An *in vivo* micronucleus test on mouse bone marrow cells was negative (Shelby et al. 1993[15]) and another one was positive (Mohtashamipur et al. 1987[16]). A micronucleus test on peripheral blood reticulocytes of mice gave negative result (Kim & Ryu, 2007[17]).

Two somatic mutation and recombination tests were performed on Drosophila melanogaster with 1,2-dichlorobenzene. It proved to be negative in one test and weakly positive in the other one (Vogel & Nivard, 1993[18]).

1,2-dichlorobenzene could bind *in vitro* to calf thymus DNA and *in vivo* to DNA in liver, kidney, lung and stomach of rats and mice. The covalent binding index to liver DNA was typical of mutagenic carcinogens classified as weak initiators (Colacci et al. 1990[19]).

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[8] Masumori S, Itakura N, Kikuchi M, Kajihara R, Suzuki Y. (2001b). *In vitro* chromosomal aberration test of o-dichlorobenzene on cultured Chinese hamster cells. Toxicity Testing Reports of Environmental Chemicals. 8:333-336.

[9] Myhr BC, Caspary WJ. (1991). Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: results for 31 coded compounds in the National Toxicology Program. Environmental and Molecular Mutagenesis. 18: 51-83.

[10] Kim Y-J and Ryu J-C. (2007). Evaluation of the Genetic Toxicity of Synthetic Chemical (XVII) – *In vitro* Mouse Lymphoma Assay and *in vitro* Supravital Micronucleus Assay with 1,2-Dichlorobenzene. Molecular and Cellular Toxicology, 3: 113-118.

[11] Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B (1987). Prediction of chemical carcinogenicity in rodents from *in vivo* genetic toxicity assays. Science 236: 933-941.

[12] Bioassay Systems Corp. (1984). *In vitro* gene mutation assay ((HGPRT locus) in cultured Chinese hamster ovary cells on ortho-dichlorobenzene. EPA/OTS Doc No. 40-8420664, 1-23.

[13] Reustle, JA. and Scriber, HE. (1979). o-Dichlorobenzene: Myelotoxicity and cytogenet study in rats. Report of the Rhom and Haas Company, Pennsylvania: EPA/OTS Doc. No. 878212182, 1-71.

[14] Kitchin, KT., Brown, JL. and Kulkarni, AP. (1992). Predictive assay for rodent carcinogenicity using *in vivo* biochemical parameters: operational characteristics and complementary. Mut Res, 266:253-272.

[15] Shelby, MD., Erexson, GL., Hook, GJ. and Tice, RR. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 94 chemicals. Environ Mole Muta, 21:160-179.

[16] Mohtashamipur, E., Triebel, R., Straeter, H. and Norpoth, K. (1987). The bone marrow clastogenicity of eight halogeneted benzenes in male NMRI mice. Mutagenesis, 2:111-113.

[17] Kim Y-J and Ryu J-C. (2007). Evaluation of the Genetic Toxicity of Synthetic Chemical (XVII) – *In vitro* Mouse Lymphoma Assay and *in vitro* Supravital Micronucleus Assay with 1,2-Dichlorobenzene. Molecular and Cellular Toxicology, 3: 113-118.

[18] Vogel, EW and Nivard, MJM. (1993). Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis, 8:57-81.

[19] Colacci A, Bartoli S, Bonora B, Niero A, Silingardi P, Grilli, S. (1990). *In vivo* and *in vitro* interaction of 1,2-dichlorobenzene with nucleic acids and proteins of mice and rats. Tumori, 76: 339-344.

Chromosome studies done in humans who were exposed to vapours of 1,2-dichlorobenzene showed that exposure increased the chromosomal aberrations in the peripheral blood cells significantly. There was a significant reduction of chromosomal aberrations six months later, but some aberrations were still present. There are no data about gaps, whether they were excluded or included during the analysis, but the reduction of aberrations shows that the exposure caused a genotoxic effect. There are no data about the purity of 1,2-dichlorobenzene, therefore one cannot exclude the possibility that the genotoxic effect was induced by clastogenic impurities (Zapata-Gayon et al. 1982[20]).

The Registrant(s) are of the opinion that a classification is not justified, based upon the opinion of different expert committees and the uncertainty of some mutagenicity studies which gave positive results. The Registrant(s) concluded: "*The positive micronucleus test conducted by Mohtashamipur et al in 1987 could not be reproduced in a more recent, well-conducted study (Shelby et al 1993), the in vitro DNA binding assay measured only binding but did not identify DNA adducts and little confidence can be attributed to the chromosomal aberrations in peripheral blood leukocytes reported from accidentally exposed humans.*"

However, the negative result of the more recent *in vivo* micronucleus test (Shelby et al. 1993) does not set aside the positive result of the other *in vivo* micronucleus test (Mohtashamipur et al. 1987). It is notable, that the doses in Mohtashamipur's test were considerably higher than that in Shelby's test. The *in vivo* and *in vitro* binding to DNA is a genotoxic effect without identifying the adduct (Colacci et al. 1990). The chromosomal aberrations in peripheral blood leukocytes of exposed humans (Zapata-Gayon et al. 1982) could be caused by 1,2-dichlorobenzene or by impurities or by both of them, but it is a real genotoxic effect too.

Consequently, ECHA concluded that the present data-set is not sufficient for classification or non-classification. A number of *in vitro* and *in vivo* mutagenicity tests were performed on 1,2-dichlorobenzene and based upon the relevant, available data the possible mutagenic properties of 1,2-dichlorobenzene were critically analysed. All these tests were reliable or at least reliable with restrictions, in most cases with no data on GLP available.

Although the majority of the tests gave negative results, 1,2-dichlorobenzene can be mutagenic or genotoxic in some types of somatic cells, and as it can bind to DNA the possibility that it is genotoxic to humans cannot be excluded. Consequently, the initial concern cannot be resolved, and further information is necessary.

The Registrant(s) proposed in their comment that, if uncertainties remain a combined *in vivo* Mammalian Erythrocyte Micronucleus test and *in vivo* rat COMET assay after sub-acute inhalation (28 days) should be conducted.

In order to clarify the above highlighted biases in the literature ECHA requests as first tier an *In vivo* Mammalian Alkaline COMET Assay (OECD 489) combined with *In vivo* Mammalian Erythrocyte Micronucleus Test (OECD 474) by oral route in rat. The reason to choose the oral route is that several *in vitro* tests were positive only after metabolic activation, which is higher after oral dosing, thus the passage through the liver appears to be crucial. Further to this, there is no specific reason to use inhalation exposure. The

[20] Zapata-Gayon, C., Zapata-Gayon, N. and Gonzalez-Angulo, A. (1982). Clastogenic chromosomal aberrations in 26 individuals accidentally exposed to ortho-dichlorobenzene vapors in the National Medical Center in Mexico City. Arch Environ Health, 37: 231-235.

most relevant organs for this endpoint and test method apart from the first site of contact are then the liver and bone marrow, for which inhalation exposure is not necessary. It should also be considered that the substance is classified as STOT SE 3 for respiratory irritation, thus inhalation exposure may lead to additional stress to the animals.

The published experimental results suggest that 1,2-dichlorobenzene can probably produce gene mutations *in vitro* in mammalian cells, but the data are contradictory. The combined Micronucleus Test and the COMET test can provide the information necessary to decide whether 1,2-dichlorobenzene is clastogenic and/or possibly mutagenic. The COMET Assay is able to detect DNA strand breaks which may lead to both gene and chromosomal mutations. The COMET Assay also provides information about the consequences of the binding of 1,2-dichlorobenzene to DNA (see Colacci et al. 1990[19]). COMET can also provide data on DNA repair and, combined with the use of relevant restriction enzymes, it can also detect DNA base oxidation. In order to address possible cytogenetic affect as well, ECHA considers the need to conduct a Micronucleus Test as well. The results of the former *in vivo* mammalian erythrocyte micronucleus tests were contradictory and there is a slight possibility that the genotoxic effect detected in exposed humans was caused by 1,2-dichlorobenzene. A well conducted new micronucleus test with appropriate dosing can provide data to clarify the uncertainties. As the COMET Assay can be combined with the Micronucleus test, according to the OECD test guideline, it is considered a time- and cost-efficient approach to combine these tests.

For the COMET Assay the following tissues shall be analysed: the liver, as it is the main metabolizing organ, the glandular stomach, as it is the site of first contact, duodenum/jejunum, as the substance may potentially reach the intestines, which consist of a more sensitive tissues, and also the bone marrow, as there are contradictory data available on this tissue. For the Micronucleus Test the bone marrow shall be analysed. ECHA considers that, with an appropriate dosing, analysing this tissue yields the best information on clastogenicity.

The tests shall be conducted in conformity with the relevant OECD Test guidelines. Animals shall be dosed with 1,2-dichlorobenzene 48, 24 and 3 hours prior to sacrifice.[21]

Tier 2a. Mammalian Spermatogonial Chromosome Aberration Test (OECD 483) (in case the Micronucleus Test is positive)

Tier 2b. Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD 488) (in case the COMET Assay is positive)

As the positive results of the Tier 1 tests may highlight further issues that need clarification, further tests may be necessary to draw final conclusions on the mutagenicity of 1,2-dichlorobenzene.

In particular, if the Micronucleus Test is positive, indicating clastogenicity, ECHA requests a mammalian spermatogonial chromosomal aberration test in order to clarify whether 1,2-dichlorobenzene causes inheritable mutation.

If the COMET Assay gives a positive result indicating the possibility of gene mutations

[21] K. Pant et al. (2015). Mutat Res Genet Toxicol Environ Mutagen. 2015 Jul; 786-788: 87-97.

beside the primary DNA lesions, ECHA requests that a TGR test is conducted. The TGR is considered necessary as it can provide data on whether the DNA breaks observed in the COMET test may lead to gene mutations. It will also provide information on mutations in germ cells. In order to get satisfactory result from all stages of spermatogenesis ECHA requests 10 weeks exposure.

The preferred animal model for both tests is the rat (in line with the test guidelines) and the route of administration shall be oral, for the reasons specified above. The tests shall be conducted in conformity with the relevant OECD Test guidelines. The dosing is to be determined by the testing laboratory.

IV. Adequate identification of the composition of the tested material

The substance identity information submitted in the registration dossiers has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

In relation to the required tests, the sample of substance used for the new studies shall have the highest possible concentration of identified impurities as specified by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested materials to be subjected to the test(s) 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 test(s) must be shared by the concerned Registrant(s).

V. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental studies the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at: https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at
http://echa.europa.eu/regulations/reach/registration/data-sharing

If ECHA is not informed of such agreement within 90 days, it shall designate one of the concerned Registrant(s) to perform the tests on behalf of all of them.

VI. 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[22] 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)

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