ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-2114300810-67-01/F Helsinki, 25 June 2015

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

For N-phenyl-N-[(trichloromethyl)thio]benzenesulphonamide, CAS No 2280-49-1 (EC No 218-915-0), 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)(d) thereof for N-phenyl-N-[(trichloromethyl)thio]benzenesulphonamide, CAS No 2280-49-1 (EC No 218-915-0), submitted by [redacted] (Registrant).

- Mammalian Erythrocyte Micronucleus Test (OECD Guideline 474),
- Prenatal Developmental Toxicity Study (OECD Guideline 414),
- Daphnia magna Reproduction Test (OECD Guideline 211).

This decision is based on the registration dossier as submitted with submission number [redacted], for the tonnage band of 100 to 1000 tonnes per year. This decision does not take into account any updates after 15 January 2015, the date upon which ECHA notified its draft decision to the Competent Authorities of the Member States pursuant to Article 51(1) of the REACH Regulation.

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 16 April 2013.

ECHA held a third party consultation for the testing proposals from 18 February 2014 until 4 April 2014. ECHA received information from third parties (see section III below).

On 15 October 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 21 November 2014 the Registrant did not provide any comments on the draft decision to ECHA.

On 15 January 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 to the draft decision were submitted.

On 20 February 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 2 March 2015 ECHA referred the draft decision to the Member State Committee.

By 23 March 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.

After discussion in the Member State Committee meeting on 20-23 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 51(6) of the REACH Regulation.

II. Testing required

A. Tests required pursuant to Article 40(3)

The Registrant shall carry out the following proposed tests pursuant to Article 40(3)(a) and 13(4) of the REACH Regulation using the indicated test methods and the registered substance subject to the present decision:

1. *In vivo* mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2; test method B.12./OECD 474);
2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: EU B.31/OECD 414) in rats or rabbits, oral route;
3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: *Daphnia magna* reproduction test, EU C.20/OECD 211).

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 requests in this decision, or to fulfil otherwise the information requirements 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 **3 July 2017** an update of the registration dossier containing the information required by this decision, including, where relevant, an update of the Chemical Safety Report. The timeline has been set to allow for sequential testing as appropriate.

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 and scientific information submitted by third parties.

Tests required pursuant to Article 40(3)

1. *In vivo* mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"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 several *in vitro* studies that show positive results. Mutagenic activity was observed, with and without metabolic activation, in two bacterial reverse mutation assays conducted according to OECD 471. Moreover, an *in vitro* mammalian cell micronucleus test (OECD 487) showed a biologically relevant increase in the frequencies of micronucleus in V79 cells treated with the test item in the absence or in the presence of a S9 mix (4 hours treatment).

An appropriate *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations is not available for the registered substance. Consequently, there is an information gap and the Registrant proposed to generate information for this endpoint.

Hence, the Registrant has submitted a testing proposal for an *in vivo* mammalian erythrocyte micronucleus test according to OECD 474 in order to further investigate the effects observed in the *in vitro* studies.

ECHA notes that this test is an appropriate test to investigate further effects on chromosomal aberrations *in vivo* as described in the ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.1. and figure R.7.7-1 (February 2014).

A Member State proposed that "the registrant should consider that in case of a negative test result of the *in vivo* micronucleus test, an *in vivo* gene mutation test should be performed to fully prove there is no mutagenic potential. In view of 3R, the registrant may consider to integrate the proposed *in vivo* micronucleus test with an *in vivo* Comet assay". In his response to this proposal for amendment, the Registrant expressed disagreement

with the proposal to combine the comet assay and the micronucleus assay. The Registrant based his opinion on the observations on gastric mucosa reported in the sub-acute toxicity study and considers that dose selection for a combined study (i.e. no local effect on the forestomach but measurable toxicity in the bone marrow) might result in non-optimal doses for both endpoints and interpretation of such a study will be difficult and/or limited. However, ECHA considers that the Registrant should consider the "Notes for consideration by the Registrant" (including the references) below which provide further information on the combination of these two assays and how to address possible confounding cytotoxicity.

b) Consideration of the information received during third party consultation

A third party recommended that an *in vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (i.e. a "Comet assay") should be performed in place of the proposed mammalian micronucleus test in order to avoid potential separate *in vivo* tests for chromosomal aberrations and gene mutations.

ECHA considers that an *in vivo* micronucleus test should be the preferred study for investigating cytogenetic damages. ECHA notes that a positive result was obtained in the *in vitro* micronucleus test. Therefore the rodent micronucleus test is deemed appropriate to best address clastogenic and aneugenic potential (see ECHA Guidance document on information requirements and chemical safety assessment R.7a, chapter R.7.7.6.3).

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: i*n vivo* mammalian erythrocyte micronucleus test (test method: EU B.12./OECD 474).

Notes for consideration by the Registrant

Due to the nature of the substance, the Registrant is reminded that, according to paragraph 10 of the OECD 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 474, a test chemical is considered clearly negative 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.

The Registrant is reminded that according to the column 2 of section 8.4. of Annex IX 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". ECHA notes that the examination of gonadal cells would optimize the use of animals. Positive results in whole gonad that contains a mixture of somatic and germ cells are not necessarily reflective of germ cell damage, but they indicate that tested substance(s) and/or its metabolites have reached the gonad and caused genotoxic effects. This type of evidence may still be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

A Member State proposed that the registrant should consider that in case of a negative

test result of the *in vivo* micronucleus test, an *in vivo* gene mutation test should be performed to fully prove there is no mutagenic potential. In view of the 3Rs principle (replacement, reduction, refinement of experimental studies in vertebrate animals), the registrant may consider to integrate the proposed *in vivo* micronucleus test with an *in vivo* Comet assay[1,2,3].

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

a) Examination of the testing proposal
Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. 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 pre-natal developmental toxicity study according to EU B.31/OECD 414 to investigate the potential developmenta toxicity of the registered substance.

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 8.7.2. of the REACH Regulation.

The Registrant did not specify the species to be used for testing. He did not specify the route for testing either. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species, and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat or the rabbit as a first species to be used.

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.

A third party noted that a prenatal developmental toxicity study would not be required if the substance were to be classified as a germ cell mutagen, and if appropriate risk measurements were to be implemented. He therefore recommended that a sequential testing should be applied, and that mutagenicity should be investigated further before concluding whether the prenatal developmental toxicity study is necessary.

ECHA acknowledges the third party comment but notes that it is the Registrant's responsibility to consider and justify in the registration dossier any adaptation of the

[1] Vasquez, M.Z. (2010). Combining the in vivo comet and micronucleus assays: a practical approach to genotoxicity testing and data interpretation. *Mutagenesis* 25 (2), 187-19.
[2] Recio L *et al*, (2010), Dose-response assessment of four genotoxic chemicals in a combined mouse and rat micronucleus (MN) and Comet assay protocol, *J. Toxicol. Sci.* 35:149-62.
[3] Bowen DE, *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. *Mutat Res* 722: 7-19.

information requirements in accordance with Annex IX, Section 8.7., column 2, second indent. This adaptation specifies that in case the substance is known to be a germ cell mutagen (which correspond to a classification as germ cell mutagen category 1A or 1B) and appropriate risk management measures are implemented, the pre-natal developmental toxicity study does not need to be conducted. However, the substance is so far not classified as germ cell mutagen.

ECHA further notes that the deadline to submit the requested information is set so that it allows sequential testing. Therefore the Registrant will have the possibility to perform the mutagenicity test first.

Moreover, ECHA notes that although a positive *in vivo* Comet assay may contribute to a classification as germ cell mutagen, this test is usually not sufficient on its own for classification as germ cell mutagen category 1B.

c) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is requested to carry out the proposed study with the registered substance subject to the present decision: Pre-natal developmental toxicity study in rats or rabbits, oral route (test method: EU B.31/OECD 414).

3. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.)

a) Examination of the testing proposal

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

"Long-term toxicity testing on aquatic invertebrates" is a standard information requirement as laid down in Annex IX, Section 9.1.5. 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 long-term toxicity testing on aquatic invertebrates, *Daphnia magna* reproduction test (OECD 211) with the following justification:

"*Chronic daphnia study is proposed as the substance has a very low water solubility and there are no effects from acute studies that are relevant for the risk assessment. Despite the low water solubility and the fact that the low amounts of the substance which are dissolved are expected to be readily hydrolysed it could be that chronic exposure leads to effects that have to be considered in the assessment.*
Therefore, a chronic study with daphnia according to OECD 211 is planned and will be performed when approved by the competent European authority ECHA."

ECHA considers that the proposed study is appropriate to fulfil the information requirement of Annex IX, Section 9.1.5. of the REACH Regulation.

According to ECHA *Guidance on information requirements and chemical safety assessment* (version 1.2., November 2012), Chapter R7b (Section R.7.8.5 including Figure R.7.8-4), if based on acute aquatic toxicity data neither fish nor invertebrates are shown to be substantially more sensitive, long-term studies may be required on both. There were no

indications in the dossier from the short-term toxicity studies on aquatic species that the fish would be substantially more sensitive than aquatic invertebrates.

In such case, according to the integrated testing strategy, the *Daphnia* study is to be conducted first. If based on the results of the long-term *Daphnia* study no risks are predicted, no long-term fish testing may need to be conducted. However, if a risk is indicated, long-term fish testing may need to be conducted.

b) Outcome

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed study using the registered substance subject to the present decision: Long-term toxicity testing on aquatic invertebrates (Annex IX, 9.1.5.; test method: *Daphnia magna* reproduction test, EU C.20/OECD 211).

Notes for consideration by the Registrant

Once results of the proposed test on long-term toxicity to aquatic invertebrates are available, the Registrant shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation. If the revised chemical safety assessment indicates the need to investigate further the effects on aquatic organisms, the Registrant shall submit a testing proposal for a long-term toxicity test on fish in order to fulfil the standard information requirement of Annex IX, 9.1.6. If the Registrant comes to the conclusion that no further investigation of effects on aquatic organisms is required, he shall update his technical dossier by clearly stating the reasons for adapting the standard information requirement of Annex IX, 9.1.6.

Due to the low solubility of the substance in water, the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6 and the ECHA Guidance, Chapter R7b, table R. 7.8-3 summarising aquatic toxicity testing of difficult substances should be consulted by the Registrant for choosing the design of the requested long-term ecotoxicity tests and for calculation and expression of the result of this test.

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. The Registrant must note, however, that this information has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

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. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

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.

[redacted]
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