~~Confidential~~

Helsinki, 30 September 2021

Addressees
Registrants of JS_74-31-7 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
16/03/2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: N,N'-diphenyl-p-phenylenediamine
EC number: 200-806-4
CAS number: 74-31-7

Decision number: Please refer to the REACH-IT message which delivered this communication (in format TPE-D-XXXXXXXXXX-XX-XX/F)

DECISION ON TESTING PROPOSAL(S)

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***9 October 2023***.

The requested information must be generated using the Substance unless otherwise specified.

A. Information required from the Registrants subject to Annex VIII of REACH

1. *In vivo* genetic toxicity test:

a) In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum;

OR

b) Transgenic rodent somatic and germ cell gene mutation assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 488 from 2020) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in transgenic mice or rats, oral route. For the TGR assay the following tissues shall be analysed: liver and glandular stomach; duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

2. Extended one-generation reproductive toxicity study (Annex VIII, Section 8.7.3.; test method: OECD TG 443) in rats, oral route specified as follows:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation.

You must report the study performed according to the above specifications. Any expansions of the study design must be scientifically justified.

Reasons for the request(s) are explained in the following appendix entitled "Reasons to request information required under Annexes VIII of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH, the information specified in Annexes VIII to REACH, for registration at 10-100 tpa.

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Approved[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[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.

Appendix A: Reasons to request information required under Annex VIII of REACH

This decision is based on the examination of the testing proposals you submitted.

1. In vivo genetic toxicity test

Under Annex VIII Section 8.4., column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, the *in vitro* cytogenicity tests and the *in vitro* gene mutation study in mammalian cells which raise the concerns for gene mutations and for chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

Therefore, you submitted two testing proposals for an *in vivo* mammalian erythrocyte micronucleus test and for a transgenic rodent somatic and germ cell gene mutation assay, both tests to be performed with the Substance. Furthermore, you are proposing a tiered testing approach: "*First, the micronucleus test should be conducted. If this assay is positive and allows a clear conclusion regarding classification and labelling, the transgenic rodent gene mutation assay is obsolete and can be omitted. If the micronucleus test is negative, the transgenic rodent gene mutation assay should be performed as a second step to gain clarity regarding the mutagenic potential of the substance*."

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity *in vivo*. ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA agrees that appropriate *in vivo* follow up genotoxicity studies are necessary to address the concerns identified *in vitro.*

1.1 Test selection

ECHA notes that the proposed tests are appropriate to investigate either gene mutations or chromosomal aberrations *in vivo*.

More specifically, according to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) is suitable to follow up a positive *in vitro* result on chromosomal aberration while the transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) is suitable to follow up a *positive in vitro* result on gene mutation.

ECHA notes that according to the ECHA Guidance there is also the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) which is a genotoxicity indicator test, that is suitable to follow up the positive *in vitro* results for both chromosomal aberration and gene mutation. However, we also note that the MN test is a mutagenicity test that provides evidence on *in vivo* chromosomal mutagenicity, as this study detects both structural and numerical chromosomal aberrations.

Moreover, ECHA notes that according to the ECHA Guidance it is possible to combine the tests into a single *in vivo* study, and thereby save on resources and numbers of animals used. Such

combination will provide information faster than in a tiered approach, in line with the REACH objective of protection of human health and will address both identified concerns.

Therefore, instead of the tiered approach you proposed, which would, for example, not address the gene mutation concern in case of positive results in the MN test, ECHA requests you to combine the following studies: either a) comet assay with MN test, or b) TGR with MN test. As noted above, the combined study can help to reduce the number of tests performed and the number of animals used, as well as to save time, while addressing both chromosomal aberration and gene mutation concerns.

1.2 Test design

You proposed testing in the mouse. You did not specify the route for testing.

a) Comet assay combined with MN test

According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combined test (OECD TG 489 and OECD TG 474) must be performed in rats.

Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

The combination of OECD TGs 489 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the dosing, and tissue sampling for the comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al*. 2011[2]).

Germ cells
You may consider to collect the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells. 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.

b) TGR assay combined with MN test

According to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally. Therefore, the combined test

[2] Bowen D.E. 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. Mutation Research 722 7–19

(OECD TG 488 and OECD TG 474) must be performed in either transgenic mice or rats using the oral route.

Based on the recent update[3] of OECD TG 488, you are requested to follow the new 28+28d regimen, as it permits the testing of mutations in somatic tissues as well as in tubule germ cells from the same animals. This updated version provides for a transitional period for the new version. However, ECHA is aware that testing according to the updated OECD TG is already available from CROs and the new study design would provide meaningful germ cell data, so this decision requires the application of the new version.

According to the test method OECD TG 488, the test must be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and duodenum as rapidly proliferating tissue and site of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

The combination of OECD TGs 488 and 474 should not impair the validity of and the results from each individual study. Careful consideration should be given to the choice of the top dose, the treatment schedule in relation to the tissue sampling for the TGR analysis and for the mammalian erythrocyte micronucleus test. Moreover, to ensure the validation of the combination study, concurrent positive control animals must be used, even if the laboratory has demonstrated competency (see OECD TG 488, para. 24) in the TGR assay, as a stand-alone test.

Germ cells
You may consider to collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, to decide on the need for assessment of mutation frequency in the collected germ cells. 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.

1.3 Outcome

Under Article 40(3)(b) your testing proposals are accepted under modified conditions and you are requested to conduct the test with the Substance, as specified above.

2. Extended one-generation reproductive toxicity study

According to Annex VIII, 8.7.1., Column 2, at the tonnage level of 10 to 100 tonnes per annum, the Registrant may propose an extended one-generation reproductive toxicity study (EOGRTS, OECD TG 443) (Annex IX, section 8.7.3) instead of a screening study in cases

[3] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66.

where there are serious concerns about the potential for adverse effects on fertility or development.

Pursuant to Article 12(1) and Annex VI of the REACH Regulation the standard information requirements listed in Annex VII to X of the REACH Regulation are considered minimum requirements. Annex VI, step 4 of the 'Guidance note on fulfilling the requirements of Annexes VI to XI' provides that the rules set out in Annexes VII to XI may require certain tests to be undertaken earlier than or in addition to the standard requirements. Furthermore, in accordance with Annex I of the REACH Regulation, certain additional information may have to be generated if it is necessary for producing the chemical safety report (CSR). According to the last subparagraph of Section 0.5. of Annex I of REACH, if the manufacturer or importer considers that further information is necessary for producing his CSR and that this information can only be obtained by performing tests in accordance with Annex IX and X, he shall submit a proposal for a testing strategy, explaining why he considers that additional information is necessary and record this in the CSR under the appropriate heading.

This means that when justified, higher tier/further studies may be conducted for substances where the tonnage level would not normally require this as a standard requirement. In order to understand the toxicological properties of the registered substance in light of the adverse effects observed, it is necessary to investigate further so that appropriate risk management measures can be put in place and safe use of the substance can be ensured.

Your dossier contains a screening study (Matsumoto *et al*., 2013; OECD TG 421) with the Substance. In this study, gestation times were significantly elongated in female rats in the MD and HD groups. No clinical signs were observed in pregnant females until day 22, after which delayed onset of parturition and severe maternal toxicity including mortality could be observed. In comparison in a 28-day study by Matsumoto et al, there were no significant effects up to the limit dose of 1000 mg/kg. Consequently, you have self-classified the substance as Repr. 2 (H361; Fertility).

Based on these effects, you have identified a serious concern. To clarify the reproductive/developmental toxicity profile of the Substance, and the most appropriate classification, you propose to conduct an EOGRTS.

ECHA considers that the potential adverse effect on fertility, in specific prolonged gestation periods, reported in the screening study (OECD TG 421) raises serious concerns about the potential for adverse effects on reproduction (e.g. significant/severe effects). In addition to confirming the already observed effects from the screening studies, EOGRTS may show more effects related to the modes of action because more sensitive parameters are investigated with higher statistical power. This will lead to more reliable NOAEL values and more reliable hazard classification. ECHA considers that the Substance shows concerning toxicological properties which need to be further investigated in order to conclude on risk assessment and classification based on a more definitive study design and thus in order to produce the CSR. This is necessary for appropriate risk management measures.

2.1 Information provided to fulfil the information requirement

You have identified a need to perform an EOGRTS and submitted a testing proposal for an EOGRTS according to OECD TG 443 with the Substance.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Toxicity to reproduction. You provided your considerations concluding that there were no alternative methods which could be used to adapt the information

requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA has evaluated your proposal to perform an EOGRTS according to OECD TG 443 at Annex VIII.

ECHA agrees that an EOGRTS is necessary to address the identified concerns in relation with reproductive/developmental toxicity.

2.2 Specification of the study design

Species and route selection

You proposed testing in the rat. ECHA agrees with your proposal because the rat is the species preferred by OECD TG 443.

You proposed testing by oral route. ECHA agrees with your proposal. The oral route is the most appropriate route of administration to investigate reproductive toxicity[4].

Pre-mating exposure duration and dose-level setting

You proposed two weeks pre-mating exposure duration. However, ten weeks pre-mating exposure duration is required because there is no substance specific information in the dossier supporting shorter pre-mating exposure duration (ECHA Guidance R.7a, Appendix R.7.6-3).

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level must aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects, with the other cohorts being tested at the same dose levels. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range-finding studies) are reported with the main study.

You must provide a justification with your study report that demonstrate that the dose level selection meets the conditions described above.

Cohorts 1A and 1B

Cohorts 1A and 1B belong to the basic study design and shall be included.

2.3 Outcome

Your testing proposal is accepted under Article 40(3)(b) and you are requested to conduct the test with the Substance, as specified above.

Further expansion of the study design

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by

[4] ECHA Guidance R.7a, Section R.7.6.2.3.2.

including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex IX/X. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance R.7a, Section R.7.6.

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

1. Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

2. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[5].

B. Test material

1. Selection of the Test material(s)

   The Test material used to generate the new data must be selected taking into account the following:
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test material must contain that constituent/ impurity.

2. Information on the Test material needed in the updated dossier

   - You must report the composition of the Test material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[6].

[5] https://echa.europa.eu/practical-guides
[6] https://echa.europa.eu/manuals

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 22 November 2019.

ECHA held a third party consultation for the testing proposal(s) from 27 January 2020 until 12 March 2020. ECHA did not receive information from third parties.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the request(s).

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments and referred the modified draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s) by the set deadline.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-75 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix D: List of references - ECHA Guidance[7] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[8]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)[8]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents[9]

[7] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[8] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[9] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.