EUROPEAN CHEMICALS AGENCY

Helsinki, 17 June 2020

Addressees
Registrant(s) of JS 13680-35-8 / 237-185-4 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
15 December 2017

Registered substance subject to this decision ("the Substance")
Substance name: 4,4'-methylenebis[2,6-diethylaniline]
EC number: 237-185-4
CAS number: 13680-35-8

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***22 September 2022***.

Requested information must be generated using the Substance unless otherwise specified.

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

1. Partition coefficient n-octanol/water (Annex VII, Section 7.8.; using an appropriate test method)

2. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: EU B.13/14. /OECD TG 471) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102

B. Information required from all the Registrants subject to Annex IX of REACH

1. Pre-natal developmental toxicity study in a second species (triggered by Annex IX, Section 8.7.2., column 2; test method: OECD TG 414) by oral route, in a second species (rabbit)

Reasons for the request(s) are explained in the following appendices:

- Appendices entitled "Reasons to request information required under Annexes VII to X 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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;

- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;

You are only required to share the costs of information that you must submit to fulfil your information requirements.

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".

The studies relating to biodegradation and bioaccumulation are necessary for the PBT assessment. These endpoints are not addressed in this decision, but ECHA reserves the right to request any further information in a compliance check at a later stage once the information requested in this decision is available to ECHA.

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

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 VII of REACH

1. Partition coefficient n-octanol/water

Partition coefficient n-octanol/water is a standard information requirement in Annex VII to REACH.

You have provided a key study according to OECD TG 117 (Determination of the Partition Coefficient of LZ 596, [redacted] 2014) with the Substance.

EU A.8 and OECD TG 117 establish the requirements for the data to be reported for a partition coefficient study.

For a HPLC method, especially the following is required:

- reference substances: purity, structural formula and CAS number;
- pH;
- elution profiles (chromatograms);
- deadtime and how it was measured;
- retention data and literature log Pow values for reference substances used in calibration;
- details on fitted regression line (log k versus log Pow) and the correlation coefficient of the line including confidence intervals;
- average retention data and interpolated log Pow value for the test substance;
- log Pow values relative to area % of the log Pow peak;
- calculation using a regression line;
- calculated weighted average log Pow values, when appropriate.

You have not provided the details of the provided study as listed above, and therefore, the provided study does not fulfil the information requirement.

In your comments to the draft decision, you provided further information on the study provided in your dossier. Specifically, you provided:

- reference substances: purity, structural formula and CAS number;
- a calibration equation with correlation coefficient and confidence intervals;
- you indicated that pH could not be measured, and you assume that ionisation would not occur due to low water solubility of the Substance (0.44 mg/l), and in any case it would not be technically feasible to determine the dissociation of the Substance; and
- you concluded that a new study does not need to be conducted.

ECHA has evaluated the provided information and identified the following issues:

- While you provided further information regarding the existing study, you did not provide the elution chromatograms, retention data and literature logKow values for the reference substances, retention data for the Substance or log Pow values relative to area % of the log Pow peak, as required by the OECD TG 117.
- Even though the Substance may be poorly water soluble, ionisation still happens in aqueous solution, due to the Substance having ionisable groups. However, ECHA acknowledges that measuring the dissociation constant for the Substance may be technically not feasible.

Therefore, the provided study together with the additional information provided in your comments does not fulfil the information requirement.

ECHA understands that you are willing to provide a full or partial study report, together with a complete (robust) study summary, which may fulfil the information requirement for this endpoint. However, as all the necessary details of the study are not available for assessment, no conclusion on the validity of the study can be made based on the information provided in your dossier and your comments.

You are reminded that the ionisation of the substance can have a marked effect on this property and you are requested to consider ionisation when performing the requested test.[2]

2. In vitro gene mutation study in bacteria

An *in vitro* gene mutation study in bacteria is a standard information requirement in Annex VII to REACH.

You have provided a key study in your dossier:

i. Ames study ([redacted] 1983) with the following strains, *S. typhimurium* TA 1538, TA 1535, TA 1537, TA 98 and TA 100 which all gave negative results.

ECHA has assessed this information and identified the following issue(s):

To fulfil the information requirement, the study has to meet the requirements of OECD TG 471 (1997). One of the key parameters of this test guideline includes:

a) The test must be performed with 5 strains: four strains of *S. typhimurium* (TA98; TA100; TA1535; TA1537 or TA97a or TA97) and one strain which is either *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101)

The reported data for the study you have provided did not include:

- results for the required fifth strain, *S. typhimurium* TA102 or *E. coli* WP2 uvrA or *E. coli* WP2 uvrA (pKM101).

The information provided does not cover one of the key parameters required by OECD TG 471.

In your comments to the draft decision you have agreed to perform an additional test with the missing fifth strain (E.coli WP2uvrA or E.coli WP2uvrA) in order to supplement the existing available information.

ECHA acknowledges your agreement in your comments.

Based on the above, the information you provided does not fulfil the information requirement.

Information on study design

An *In vitro* gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: EU B.13/14. /OECD TG 471) must be performed using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102.

[2] ECHA Guidance R.7a, Section R.7.1.8.5.

Appendix B: Reasons to request information required under Annex IX of REACH

1. Pre-natal developmental toxicity study in a second species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH. Annex IX, Section 8.7.2., column 2 provides that the decision on the need to perform a PNDT study on a second species at a tonnage level of 100 to 1000 tonnes per year should be based on the outcome of the PNDT study on a first species and all other relevant and available data.

You have provided:

1. A PNDT study ([redacted] 2016) in rats conducted according to OECD TG 414 with the Substance.

ECHA has assessed this information and identified the following issue(s):

As already mentioned above, a PNDT study on a second species is needed, if there is a concern for developmental toxicity based on the results from the PNDT study on a first species and other relevant data.

You consider that no developmental toxicity was observed in the available study.

You claim in your dossier: "*The Substance did not result in external and skeletal abnormalities or visceral malformations. The slightly higher incidence of visceral variation hydroureter at 50 mg/kg bw/day was judged to be without a toxicological relevance. The doses of 5 and 15 mg/kg bw/day of the Substance did not cause any maternal or fetal effects and therefore the NOAEL maternal toxicity: 15 mg/kg bw/day and NOAEL developmental toxicity: 50 mg/kg bw/day.*"

However, there is a concern based on information from a first species and taking all the available information into account as required in column 2 at Annex IX, section 8.7.2.
Contrary to what you concluded in your dossier, developmental toxicity was observed in the available study.

The study you provided shows:

a) Brain malformations
"Malformation of the brain" in one foetus in low dose and one fetus in mid dose. No further description of the nature of this malformation is provided. The incidence in the background data for misshapen cerebrum is on average 0.072% per litter, max 5.56% / litter. In the study with the Substance, the incidence is 1/24 = 4.2%. This is slightly below the maximum value from the control data, but it must be noted that the control data is from another laboratory and from another rat strain and therefore it is not appropriate 'historical control data' for this study. Furthermore, there is no detailed information on possible resorptions in the high dose group, i.e. there may have been similar malformations in the high dose, which were masked by resorptions.

In your comments to the draft decision (brain malformations) you state that it is unclear as to what is precisely meant by "there is no detailed information on possible resorptions in the high dose group". You explain that the total resorptions can be calculated as the sum of the reported early and late embryonic deaths and claim that there is no difference in the incidence of resorptions between treated groups. You

conclude that this is not a masking of effect by embryonic loss but the lack of observed brain malformations in the high-dose group represents a lack of effect. Your comments also stress that only one foetus was affected in each of the low- and mid-dose groups and explain that the effect is binary; either it does occur, or it does not occur. Thus, one affected foetus is the minimum change above zero that can be recorded, irrespective of group size and litter size and that the recorded observation of these findings in the low- and mid-dose groups represents a spontaneous event unrelated to treatment, as confirmed by the lack of effect in the high-dose group.

Firstly, the information you provided in your comments, shows no significant increase in resorptions, nevertheless resorptions did occur in all of the dose groups. In addition, one dam from the high-dose group had complete early litter loss. Based on the available information it appears that the masking of additional effects cannot be ruled out.
The incidence of the brain malformations is the lowest possible that can be detected, as you pointed in your comments. However, the severity of these brain malformations has not been reported and the uncertainty as to whether the brain malformations occurred in the litter which was lost in the dam of the high dose group raises a concern for developmental toxicity.

b) Statistically significant increase in the incidence of bilateral hydroureter
"Statistically significant increase was indicated in the incidence of fetuses with variations and abnormalities due to the increase of fetuses with bilateral hydroureter in the 50 mg/kg bw/day group". According to the control data provided, incidence of hydroureter is on average 1.1% per litter (max 21.05% / litter). In the study with the Substance, it is reported that *"The percentage of bilateral hydroureter was not higher than 7%".* However, again, the problems with the 'historical control data' as explained above under issue a) must be noted. Furthermore, the increase in the incidence of this effect seems to be statistically significant.

In your comments to the draft decision you state that the increase in incidence of the variation bilateral hydroureter in the high-dose group is statistically significant. In addition the dams of the high-dose group showed a statistically significant reduction in body weight gain (corrected for the weight of the gravid uterus, over the course of the study). Compared to the concurrent control group, the corrected weight gain in the high-dose group was reduced by almost 20% (35.1 g vs. 42.5 g).

You also stated that the historical control data for the SD rat shows the background incidence of hydroureter to decrease over the period of late gestation in the rat (MARTA, 1993). On gestation day (GD) 19 the litter incidence (mean±SD) was 1.11±3.43%, while on GD20 and GD21 it was 1.56±4.02% and 0.12±0.72% respectively. You state that this *"indicates along with hydronephrosis (Woo, 1972), hydroureter is not only spontaneously resolved, but that is associated with the overall maturation of the foetus".* You therefore conclude that it is plausible that the increased incidence of the variation hydroureter in the high-dose group reflects slight delays in pre-natal maturation associated as a secondary response to reduced maternal weight gain.

ECHA understands that you believe that the foetal effects (significant increase in incidence of bilateral hydroureter) observed are not treatment related but an indication of a delay in fetal maturation secondary to maternal toxicity (significant reduction in maternal body weight gain).

As you have indicated in your response, *"hydroureter [.....] is associated with the overall maturation of the foetus".* However, no delays in ossification or other effects indicating delay in foetal maturation were reported at external, visceral and/or skeletal examination of foetuses and the foetal body weights were not affected by treatment. Therefore, there is no indication that the reduction in maternal body weight gain led to delayed foetal maturation and that the increased incidence reported in bilateral hydroureter in the high dose group could be explained solely by reduced maternal body weight gain.

Therefore based on the available information ECHA considers that there remains a cause for concern.

Furthermore, the historical control data (MARTA, 1993)[3] you have referred to is based on a different rat strain (Sprague-Dawley) compared to the provided PNDT study which is conducted with Wistar rats. Historical control data should always reflect the same strain, same laboratory, and the same study design/duration, collected from fairly recent studies (± 2 years). Therefore, the provided historical data does not provide relevant information for the study under consideration.

In your comments to the draft decision, you state that *"The assessment of the prenatal developmental toxicity study in rats ([redacted] 2016), concludes that treatment-related adverse effects on development occurred. The reassessment addresses the basis for that conclusion, and finds that there no primary treatment-related effects upon development"* and therefore *a "A test in a second species is unwarranted."*

According to the ECHA Guidance R.7a states *"A study on a second species might be necessary if the available data contain triggers for prenatal developmental toxicity. For example, performance of a prenatal developmental toxicity study in a second species may be justified if developmental effects that are not sufficient to meet classification criteria to Category 1B reproductive toxicant (but maybe sufficient to Category 2 reproductive toxicant) were observed in the prenatal developmental toxicity study with the first species."*

As explained above, the results of the PNDT study in rats (Study 1), i.e. the brain malformations and the statistically significant increase in the incidence of bilateral hydroureter, remain a concern for developmental toxicity and warrants further investigations.

As the condition of Annex IX, section 8.7.2., column 2 is fulfilled, a pre-natal developmental toxicity study in two species is an information requirement for your registration. You have not provided an OECD TG 414 study on a second species.

Therefore based on the above, the information you provided does not fulfil the information requirement.

Information on study design

Species/strain

[3] MARTA, Middle Atlantic Reproduction and Teratology Association (1993). Historical Control Data for Development and Reproductive Toxicity Studies using the CrI:CD® BR Rat. Accessed 24.01.2020 from https://www.criver.com/sites/default/files/resources/rm_rm_r_tox_studies_crlcd_br_rat.pdf

A PNDT study according to the OECD TG 414 study should be performed in the rabbit or rat as the preferred species. The test in the first species was carried out by using a rodent species (rat). Therefore, a PNDT study in a second species must be performed in the rabbit as preferred non-rodent species.

Administration route

The study shall be performed with oral[4] administration of the Substance.

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

Appendix C: 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

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

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 variation in compositions reported by all members of the joint submission,
   - 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 D: Procedure

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

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

The compliance check was initiated on 20 August 2019.

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

ECHA took into account your comments and amended the request(s).

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix E: 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 multiconstituent 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.

[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

OECD Guidance documents[9]
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.

[9] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix F: 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]
[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.