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Helsinki, 06 February 2018

Substance name: For 4,4'-methylenebis[N,N-bis(2,3-epoxypropyl)aniline]
EC number: 249-204-3
CAS number: 28768-32-3
Date of latest submission(s) considered[1]: 6 April 2017
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressee(s): Registrant(s)[2] of 4,4'-methylenebis[N,N-bis(2,3-epoxypropyl)aniline abbreviated TGMDA in the following.

DECISION ON SUBSTANCE EVALUATION

Based on Article 46(3) of the REACH Regulation (Regulation (EC) No 1907/2006), you are requested to submit the following information on the registered substance 4,4'-methylenebis[N,N-bis(2,3-epoxypropyl)aniline] (monoconstituent TGMDA, CAS 28768-32-3) or the UVCB TGMDA (CAS 28390-91-2) with the composition specified in the registration dossier:

Human health endpoint mutagenicity

Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays in mouse or rat by oral gavage (EU B.58./OECD 488) following a 28-day exposure with a subsequent 49 days (mouse), or 70 days (rat) sampling period. Male germ cells from the cauda epididymis shall be sampled and analysed.

Dosing shall be done by oral gavage daily in a freshly prepared test solution using an appropriate vehicle.

You have to provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the chemical safety report by **13 November 2019**.

The evaluating Member State Competent Authority (MSCA) must have access to the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are, based on experience, not always available in robust study summaries only.

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[1] This decision is based on the registration dossier(s) on the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

[2] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

The deadline takes into account the time that you may need to agree on which of the registrant(s) will perform the required tests.

The reasons of this decision and any further test specifications are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This appendix is confidential and not included in the public version of this decision.

Who performs the testing?

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

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 a suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

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

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Appendix 1: Reasons

Based on the evaluation of all relevant information submitted in a dossier update on 4,4'-methylenebis[N,N-bis(2,3-epoxypropyl)aniline] and other relevant available information, ECHA concludes that as a follow-up further information is required to enable the evaluating Member State competent authority to complete the evaluation of whether the substance constitutes a risk to human health.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested to clarify the concern for mutagenicity.

Note on read-across:

Experimental studies concerning the endpoint of mutagenicity have been conducted with either the monoconstituent TGMDA, (CAS 28768-32-3) or the UVCB TGMDA (CAS 28390-91-2). Limited to the present substance evaluation purpose to clarify the concern identified for mutagenicity, the MSCA has accepted the read across, from the UVCB TGMDA substance (substance of unknown or variable composition, complex reaction products or biological materials) (CAS 28390-91-2) to the monoconstituent TGMDA (CAS 28768-32-3) based on the justification document provided by you. The secondary constituents are essentially the same in both substances. However, their concentrations are slightly higher in the UVCB substance vs. the monoconstituent substance. It means that the impurities (or the intermediate reaction substances) of the monoconstituent substance are identical with the secondary constituents of the UVCB substance. The moderate difference in purity is not due to the presence of different constituents, but due to their composition ratios.

Human health endpoint Mutagenicity:

The concern(s) identified

Based on the positive results of the available *in vitro* and *in vivo* mutagenicity studies (as described in detail below) there is concern that the substance may cause gene mutations in germ cells and somatic cells. This causes a potential risk for workers. However, the available information is not conclusive for classification as germ cell mutagen category 1B. Therefore, the OECD TG 488 is needed to clarify the concern.

The substance evaluation conducted on TGMDA in 2015 showed that there was no concern for chromosomal aberrations: A reliable Mammalian Erythrocyte Micronucleus Test, OECD TG 474 from 2013, conducted according to GLP, yielded a negative result. The study was conducted with the UVCB TGMDA and the data were used by the Registrant(s) to read across to the monoconstituent TGMDA.

However, there is a concern for gene mutations *in vivo* for TGMDA. TGMDA has been shown to cause gene mutations *in vitro*: In one study from 1982, performed according to OECD TG 471, UVCB TGMDA yielded a positive result in the Salmonella strains TA 100 and TA1535 when tested up to maximal recommended concentrations. The substance was positive only with metabolic activation.

In a supplementary study from 1981 UVCB TGMDA was positive only in TA1535 with metabolic activation. No cytotoxicity was observed up to highest tested concentration.

Prior to the substance evaluation decision from 2015 no *in vivo* data of the concern for

gene mutations were available.

In the decision from 2015 you were therefore required to perform either a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays in mouse or rat by oral gavage (TGR, EU B.58./OECD 488) or an *In vivo* mammalian alkaline Comet assay in mouse or rat by oral gavage (OECD 489) as a first *in vivo* test to clarify the concern.

In order to draw a robust conclusion on germ cell mutations *in vivo*, a gene mutation test such as the transgenic rodent assay OECD 488 is needed. However, the option to perform a Comet assay OECD 489 as a first *in vivo* test investigating genotoxicity was given to the Registrant(s). If a reliable Comet assay OECD 489 yielded a negative result for TGMDA, then this would give a strong indication that TGMDA would also yield a negative result for *in vivo* gene mutations. If on the other hand a reliable Comet assay OECD 489 yielded a positive result, this would have to be followed up by considering a request for gene mutagenicity testing in germ cells. The Comet assay OECD 489 is not considered appropriate to measure DNA strand breaks in mature germ cells. In order to obtain data adequate for the purpose of classification and labelling as a germ cell mutagen category 1B, the TGR assay is needed.

Comet assay:

An *in vivo* mammalian alkaline Comet assay in rat by oral gavage (OECD 489) (version 2014) was conducted according to GLP in 2017. This test was requested as one alternative by ECHA in the decision sent to the Registrant(s) on 16 December 2015.

The test material was evaluated for its potential to induce DNA damage (DNA strand breaks) in liver, glandular stomach and duodenum cells of male Sprague-Dawley rats. Ethyl methanesulfonate (EMS) was used as positive control (20 mg/mL in 0.9% Saline). The test material used was the UVCB TGMDA substance (CAS 28390-91-2) described in the report as 4,4'-Methylenedianiline, oligomeric reaction products with 1-chloro-2,3-epoxypropane, trade name Araldite MY 9512 CH, (batch number AAE1675300). Purity was assumed to be 100%. PEG (polyethyleneglycol) 400 was selected as the vehicle. Test and/or control article formulations were administered at a dose volume of 10 mL/kg /day by oral gavage once a day on two consecutive days.

The dosing formulation of UVCB TGMDA in PEG 400 was freshly made at least once a day. Dose formulations were analyzed for stability and were stable at room temperature for at least 3 hours. The duration of dosing did not exceed the stability period of the test article in the vehicle.

Dose range finding (DRF) assay:

The dose levels tested were 500, 1000 and 2000 mg/kg/day in 3 animals per sex. Dosing was conducted on two consecutive days.

Based on the results of the DRF assay (piloerection in male and female animals and lethargy in female animals, observed only at 2000 mg/kg and day), the highest dose selected for the main study was 2000 mg/kg, which is the highest dose recommended in the OECD test guideline 489.

Liquid-chromatography tandem-mass spectrometry (LC-MS/MS) analysis:

The concentration of the test material in plasma was analyzed by a validated LC-MS/MS method. Allegedly metabolites of the test material were also analyzed by LC-MS/MS

according to the test report. For the quality control standard solution the standard recovery by spiking was 85-115% of target. Blood was collected by retro-orbital bleeding 1 hour or 3 hours after the last dose. All concentrations detected were below the lowest concentration calibration standard (25 ng/mL) and only one sample from the high dose males (sampled after 1 hour) was high enough to provide a qualitative result of ~ 14.3 ng/mL. All other samples were below the detection limit according to the study report. Another assay was then performed at shorter time intervals. The test substance was evaluated in 3 male animals. Animals were dosed with 2000 mg/kg once by oral gavage and samples were collected 15, 30, 45, 60, 120 and 180 minutes after dosing. All samples were described as being below the detection limit. LC-MS/MS was not performed for the main study.

No information was available in the study report regarding the limit of detection, limit of quantification nor the identity of investigated metabolites of UVCB TGMDA in this assay.

Main study:

The dose levels tested in the main study were 500, 1000 and 2000 mg/kg/day by oral gavage.

Six male rats per groups were used. All animals in the negative control (PEG 400) group and the test material groups were dosed on two consecutive days (Days 1 and 2). Dosing took 1-4 minutes per group. The second dose occurred approximately 21 hours after the first dose. Animals were euthanized 3-4 hours after the second dose. The positive control group was dosed once 3-4 hours prior to euthanasia on day 2. Liver, glandular stomach and duodenum were sampled. 150 cells were scored per animal and tissue using a fully validated, automated scoring system. DNA strand breaks were measured by evaluating Comet tail migration, tail moment and % tail DNA. Statistical analyses of results from test groups were performed using ANOVA followed by Dunnett's post-hoc analysis.

Each slide was also scored for hedgehogs/clouds (cells with non-existent heads, and large diffuse tails, considered to be highly damaged cells) which may indicate cytotoxicity. Histopathology was performed on liver, glandular stomach and duodenum tissue.

Results of main study:

Statistically significant increases in DNA strand breaks (% Tail DNA±SD) in liver samples were observed in the 1000 mg/kg/day (2.49±1.29%) and 2000 mg/kg/day (3.32±1.64%) dose groups when compared to the vehicle control (0.76±0.42%). This increase was dose responsive and outside the 95% control limit for the historical vehicle control (0.34±0.74). Regression analysis showed statistical significance for all three test groups.

Statistically significant increases in DNA strand breaks (% Tail DNA±SD) were also observed in glandular stomach samples in the 1000 mg/kg/day (38.65±12.57%) and 2000 mg/kg/day (35.16±7.58%) dose groups when compared to the vehicle control (19.51±11.80%). These increases were dose responsive and outside the 95% control limit for the historical vehicle control (6.98±3.62). Regression analysis showed statistical significance for all three test groups.

A statistically significant increase in DNA strand breaks (% Tail DNA±SD) in duodenum samples was observed in the 500 mg/kg/day dose group (17.78±4.79%) when

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compared to the vehicle control (4.10±2.94%). This increase was outside the 95% control limit for the historical vehicle control (4.74±3.4.34).

For all tissues, the vehicle and positive controls were in the expected ranges, with the exception of the vehicle control of the stomach.

The incidence of hedgehogs in liver samples in the low to high test substance groups was 2.5%, 2.3%, and 1.8% (vehicle control group: 2.0%, positive control group: 2.3%).

The incidence of hedgehogs in stomach samples in the low to high test substance groups was 12.8%, 34.3%, and 32.5% (vehicle control group: 14.3%, positive control group: 66.7%).

The incidence of hedgehogs in duodenum samples in the low to high test substance groups was 23.2%, 37.3%, and 46.7% (vehicle control group: 25.3%, positive control group: 40.3%).

Histopathology on liver, glandular stomach and duodenum tissue revealed no test material induced effects.

Discussion and Conclusion on the Comet assay:

The study has been conducted and evaluated according to the specifications in OECD TG 489 and is well-described. The parameter of % Tail DNA has been used to to assess the level of DNA damage as is recommended in the OECD TG 489 test guideline. All vehicle controls were within the historical control range except for the vehicle control for the stomach samples, but according to the study report PEG400 is a known compound for higher damage in the stomach. The result of UVCB TGMDA in the stomach was clearly positive for the two highest dose groups when compared to the concurrent vehicle control.

High levels of cytotoxicity may influence results in the Comet assay resulting in false positives. It is therefore recommended to investigate cytotoxicity when conducting a Comet assay. However, no single measure of cytotoxicity is recommended in the test guideline. The results of histopathology on liver, glandular stomach and duodenum tissue in the study did not give indications for cytotoxicity. Hedgehogs where scored in the study as recommended in the test guideline. Hedgehogs were previously believed to be indicators of cytotoxicity, but at present their etiology is uncertain. For liver and duodenum the incidences of hedgehogs seem unrelated to the significant increases in DNA strand breaks. For stomach samples the increases in DNA strand breaks corresponds to the increases in hedgehogs, but the the incidence of hedgehogs for the positive control was equally high for this tissue.

Based on the observations of histopathology and hedgehogs in this study it is the opinion of the evaluating MSCA that the statistically significant increases in DNA strand breaks observed in liver, stomach and duodenum have not been caused by a confounding effect of cytotoxicity.

Consideration of the Registrant(s) comments regarding the Comet assay ([redacted]):

You commented that if the metabolism of the monoconstituent and the UVCB TGMDA happens quickly (perhaps <15 minutes) as the evaluating MSCA speculates might be the case then due to the rapid metabolism the results of this Comet assay are inherently concluding that a genotoxic threshold of a TGMDA metabolite is identifiable and it does

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not induce a mutagenic response in liver, stomach or duodenum following oral dosing of mono/UVCB TGMDA at 500 mg/kg bw/d.

You noted that the statistically significant increase in % Tail DNA observed at the 500 mg/kg/day dose group of the duodenum samples was not dose responsive, at 1000 and 2000 mg/kg/day the same tissue yielded a negative response and that therefore the result was not test substance related according to you. Based on the findings of the Comet assay (█████) you believed that a threshold can be determined (500 mg/kg bw/d). You commented that this threshold dose for increases in DNA damage may be used in the evaluation of "risk" by the evaluating MSCA. Furthermore, you made specific comments regarding DNEL values for exposure.

The evaluating MSCA notes that the default assumption for genotoxic and mutagenic substances is that they have a linear dose (concentration)-response relationship and therefore no threshold below which the adverse effect can occur. In some cases experimental data have shown that certain substances and genotoxic effects (mechanisms of action) may possess non-linear or/and supposedly "thresholded" dose (concentration)-response curves. However, due to experimental variability it will generally not be possible to show whether such "thresholds" below which no effects occur really exist or whether the dose (concentration)-response curve is just shallow. Moreover, and in respect to the previously requested Comet assay, the underlying mechanism(s) of positive result in the Comet assay (█████) is not fully known and therefore no definitive conclusions can be made as to whether such mechanisms would have a threshold. The comments regarding DNEL values for exposure that you made are therefore not relevant. Furthermore, genotoxicity/mutagenicity tests like the Comet/TGR assays are not designed in order to derive no effect levels or even no observed effect level but rather to provide "yes-" or "no-" answers. The reason that high doses are used in toxicological assays such as the Comet assay is to obtain a statistically significant result using a limited number of animals in accordance with the 3R principle.

The evaluating MSCA agrees with you that no dose related effects on mortality, body weight or consumption were evident in the Comet assay (█████). It is the opinion of the evaluating MSCA that this confirms that the doses used in the study did not cause excessive systemic toxicity. The evaluating MSCA further agrees with you that the microscopic evaluation of liver, stomach and duodenum indicated that structural integrity had been maintained. As previously stated in the draft decision sent to you, it is the opinion of the evaluating MSCA that the results of histopathology on liver, glandular stomach and duodenum tissue in the study did not give indications of cytotoxicity.

Evaluation of the LC-MS/MS analysis:

The concentration of the test material in plasma were analyzed by LC-MS/MS according to the study report. A detectable level of the components of the UVCB TGMDA was only observed in one animal out of nine, and only 1 hour after the second dose of 2000 mg/kg/day (2 consecutive days of dosing). Based on this result shorter time intervals were used in a follow up, the shortest being 15 minutes after (only one) dosing. No detectable level of UVCB TGMDA was observed in these plasma samples. Because no description of the limit of detection nor limit of quantification was included in the study report the sensitivity of the performed assay is unknown; and it is therefore unknown if the absence of UVCB TGMDA UVCB in the plasma samples could be due to interference by non-specific background signals etc.

Available information relating to toxicokinetics:

It is not possible to compare the results of the LC-MS/MS to other kinetic studies of the test material since no experimental studies describing absorption, distribution, metabolism or excretion are available for either monoconstituent TGMDA or UVCB TGMDA in the registration dossiers. However, based on the physical and chemical properties of monoconstituent TGMDA or UVCB TGMDA absorption from the gastrointestinal tract is likely.: Both substances are viscous liquids with low molecular weight (i. e., <500 g/mol) and moderate log Pow values (i. e., between -1 and 4), as well as a low water solubility (i. e., around 10 mg/L).

A repeated dose toxicity study by oral gavage in rats conducted with monoconstituent TGMDA showed decreased mean body weight as well as changes in hematology (lower mean hemoglobin concentration, white blood cells, basophils and lymphocytes count as well as total protein, and albumin levels, and increased total cholesterol and inorganic phosphorus levels) at 200 mg/kg/day. At 300 mg/kg/day the numbers of immature red blood cells (reticulocytes) were decreased, which indicates that the bone marrow may have been affected by the test material. Test item-related non adverse microscopic findings were seen in the liver, mesenteric lymph nodes, stomach and duodenum. The NOAEL was considered to be 50 mg/kg/day.

No experimental studies on the metabolism of TGMDA are avaible in the registration dossier. The results from the Ames test showed that TGMDA is only clearly positive after metabolic activation, which indicates that it is not the substance itself but an active metabolite that has the genotoxic potential.

<u>Consideration of the Registrant(s) comments regarding the LC-MS/MS analysis:</u>

You disagreed with the evaluation of the LC-MS/MS analysis. According to the Registrant(s) the limit of quantification was 20 ng/mL for the LC MS/MS method, which according to you is described in both the range finding and confirmatory sections of the report.

You further commented that "As with the range finding test the confirmatory assay identified that test substance concentrations of the treated animals were below the lowest concentration calibration standard (≤20 ng/mL) thereby indicating that following oral administration and digestion, 4,4'-Methylenedianiline, oligomeric reaction products with 1- chloro-2,3-epoxypropane is not bioavailable for bodily translocation via the blood even when dosed at high doses."

The evaluating MSCA would like to call attention to the fact that it is not reported in the version of the study report made available to it that the limit of quantification is 20 ng/mL. The LC-MS/MS analysis has been evaluated as Klimisch 3, not reliable, by the evaluating MSCA. This is due to the fact that no description of the limit of detection nor limit of quantification was included in the study report making the sensitivity of the performed assay unknown; and it is therefore unknown if the absence of UVCB TGMDA in the plasma samples could be due to interference by e.g. non-specific background signals.

You commented that you would like to confirm that the samples were not analysed for metabolites.

The evaluating MSCA appreciates your clarification: It is stated in the study report that "a validated LC-MS/MS method was used to analyze the concentrations of test article or metabolites in the plasma samples", which made it unclear to the evaluating MSCA if metabolites had indeed been analyzed. This decision been amended to reflect that only the parent compound was analysed in the LC-MS/MS analysis.

<u>Conclusion on the LC-MS/MS analysis:</u>

It is the opinion of the evaluating MSCA that due to the reactivity of the unhindered epoxy groups the metabolism of monoconstituent TGMDA and UVCB TGMDA is likely to happen quickly and could happen faster than 15 minutes. Due to the limitations in reporting the LC-MS/MS analysis has been evaluated as Klimisch 3, not reliable.

<u>Consideration of the Registrant(s) comments regarding the test method:</u>

You commented that you found the reasoning regarding the request to conduct a TGR assay confusing because the request for the TG 488 in the draft decision refers to both "hazard assessment" purposes as well as to whether the substance constitutes a "risk" to human health. You further commented that in accordance with the ECHA guidance detailed in Chapter E: Risk Characterisation, Table E.3-1 the results of the comet assay and allocation of the Cat. 2. Mutagen classification result in the registered substance being allocated to the "High Hazard" band which contains synonymous risk management measures and operational conditions irrelevant of whether a substances hazard classification is a Cat. 1A, 1B or 2 mutagen and therefore the results of a TGR test would not add any further protection to workers as exposure to parent substance TGMDA is already well controlled, consequently by removing exposure to the parent molecule there is no opportunity for formation or exposure to metabolites.

ECHA notes that the prioritization of substances that are evaluated under CoRAP is done by employing a "risk-based approach" (REACH Article 44 (1)). This means that there must be a concern for hazardous properties and a potential for exposure for humans and/or the environment. TGMDA was initially chosen based on a concern for serious hazard (inherent properties of germ cell mutagenicity and carcinogenicity), and exposure (high tonnage (>100 T p.a.) and worker use). Furthermore, as stated in the draft decision, a harmonized Muta Cat. 1B classification in accordance with the CLP Regulation would elicit various downstream risk management measures according to existing EU legislation.

Your considerations concerning the "hazard bands" may refer to some specific occupational health provisions. As far as the evaluating MSCA is aware, however, there are indeed also in regard to the occupational health regulations differences in the provisions concerning somatic cell mutagens and germ cell mutagens, so the evaluating MSCA did not regard your comments to be fully correct/comprehensive.

Furthermore, as indicated above there are also other relevant regulations and risk management consequences than those under occupational health legislation, which indeed are dependent on whether the substance can be concluded to be a somatic cell or a germ cell mutagen. A harmonized Muta. Cat. 1B classification will make it possible for an EU Member State to propose to include TGMDA on the Candidate List of REACH as an initial step in the Authorisation REACH procedures. This is not the case for a substance with a harmonized Muta. Cat. 2. Classification. Finally, if the substance is a germ cell mutagen and as a consequence is added to the authorization list future uses would need to be authorized by the authorities which introduces a higher degree of certainty that indeed appropriate risk management measures are implemented to control the risk.

You further state that if a metabolite of TGMDA is the species responsible for the mutagenic response then TGMDA should not be labelled as Cat. 2 or even Cat. 1B Mutagen, but in fact it is an unknown metabolite that should be labelled/classified which is currently not the substance that is being registered or handled by workers in the industrial and manufacturing arena.

The evaluating MSCA notes in response that the requests concerning the endpoint of mutagenicity for this substance evaluation under CoRAP cover the registered substance monoconstituent TGMDA, (CAS 28768-32-3) and the read across from the UVCB TGMDA (CAS 28390-91-2), which the evaluating MSCA has accepted based on the justification document provided by the Registrant. The concern for mutagenicity includes all constituents, impurities and relevant metabolites for both TGMDA, (CAS 28768-32-3) and the UVCB TGMDA (CAS 28390-91-2).

<u>Consideration of the Registrant(s)' comments regarding the testing strategy:</u>

You commented that an OECD Standard Protocol Submission Form (SPSF) project is currently active to revise the TG 488 and that the Germ Cells workgroup of the International Life Science Institutes/Health and Environmental Sciences Institute (ILSI/HESI) Genetic Toxicology Technical Committee (GTTC) has been working towards identifying a single time point that would allow the simultaneous assessment of mutagenicity in somatic tissues and germ cells and making recommendations about potential modifications to the current recommended protocol for germ cell testing in TG 488.

The GTTC workgroup has applied a mathematical model to quantify the exposure history of germ cells collected from seminiferous tubules. According to you the results of this work show that a 28+3 day exposure of these cells does not allow the measurement of effects in cells that were spermatogonial stem cells throughout the exposure because the majority (78%) of cells isolated from the seminiferous tubules are spermatids, which according to you, the model shows would only have been exposed for an average of 1.3 days (range 0-5 days) as stem cells and 8.4 days (range 2 -11 days) as spermatogonia. Consequently, a negative result does not negate the possibility that the chemical is a germ cell mutagen. Collection of sperm from the cauda epididymis/vas deferens in a 28+3 days protocol for assessment of mutagenicity in germ cells is not useful according to the Registrant(s).

Furthermore, according to you, the model shows that if sampling is extended to 28 days (i.e. a dosing/sampling regimen of 28+28 days), germ cells isolated from the seminiferous tubules would contain spermatogonia and spermatocytes that received the majority of 28 days of exposure as stem cells or spermatogonia, whilst the majority population of spermatids would have been exposed for an average of 23 days as stem cell exposure and 4.2 days as spermatogonia.

Analysis of spermatozoa from the cauda epididymis and/or vas deferens isolated 49 or 70 days after completion of dosing ensures assessment of stem cells exposed over the entire dosing period. However, the advantage of looking at germ cells from seminiferous tubules after 28+28 days is that the mixed population of cells represents 28 days exposure during the stem cell and dividing spermatogonal stages of sperm development and also enables examination of somatic cells, without the use of additional animals.

Analysis of spermatozoa from the cauda epididymis and/or vas deferens isolated 49 or 70 days after completion of dosing ensures assessment of stem cells.

The evaluating MSCA was unable to review the conclusions of the GTTC working group because the paper describing the results had not yet become publically available. Based on your argumentation and personal communication with a member of the GTTC working group, the evaluating MSCA is of the opinion that there is scientific evidence that sampling at 28+3 days may give false negative results in germ cells because the most sensitive (mitotically active) cell populations may have not been adequately exposed to the test material, see also (O'Brien et al 2016, Tox Sci 152:363-371). This is the case both for vas deferens/cauda epididymis samples (where all the cells are at the same developmental stage) and for samples from the seminiferous tubules (mixed cell population in different developmental stages). The proposal to omit the sampling at 28+3 days of germ cells from vas deferens/cauda epididymis has therefore been accepted by the evaluating MSCA.

However, the 28+3 day sampling time is currently the recommended sampling time for somatic tissues:

As stated in the TG 488 the sampling time is a critical variable determined by the period needed for mutations to be fixed. This period is tissue-specific and depends upon the turnover time of the cell population (see paragraph 30 of TG 488). It is unknown to the evaluating MSCA at this time if a sampling time of 28+28 days (which is currently only recommended in the TG 488 for slowly proliferating tissues) would be as sensitive as 28+3 days for measuring rapidly dividing tissues such as glandular stomach and bone marrow. It is our understanding that this question will be addressed by the GTTC group.

Analysing germ cells from the seminiferous tubules at the 28+28 time point will ensure that the germ cells have been exposed during the developmental stages of stem cell as well as during mitotically dividing spermatogonial stages. Sampling sperm cells collected from the vas deferens/cauda epididymis after 49 (mouse)/70 (rat) days assesses a population of cells which have been exposed as stem cells for the entire dosing period. The evaluating MSCA is unsure which of these options would be the most sensitive for assessing germ cell mutagenicity.

It is the understanding of the evaluating MSCA that the option of sampling seminiferous tubules at the 28+28 time point and or the option of sampling vas deferens/cauda epididymis at 28+49 (mouse) or 28+70 (rat) will both be included in the revised TG 488.

You proposed a two stage approach during the conduct of the requested TGR assay:

"1) A TGR test will be conducted with an exposure of 28+3 days sampling, the following tissues shall be analysed: Male germ cells collected from the vas deferens/cauda epididymis, liver, glandular stomach and duodenum. If this test is positive it is proposed that no further testing takes place and the substance is classified as a Cat.1B mutagen and no further testing is required.

2) It the results of the 28+3 days sampling test is negative a further test will be conducted for 28 days with a 70 days post dose sampling of Male germ cells from the vas deferens/cauda epididymis to confirm a lack of effects on germ cells and therefore the registered substance TGMDA will not require labelling and classification for Mutagenicity."

The evaluating MSCA rejected your proposal of postponing germ cells testing at 28+49/70 days due to the fact that in order to conclude on germ cell mutagenicity for the purpose of obtaining an adequate harmonised classification under CLP a TGR assay

analysing relevant time points and tissues is necessary without further delay. The option to perfom a TGR assay with an exposure of 28 day+3 days sampling, which analysed somatic tissues and germ cells was already presented to you in the first final decision in 2015.

Based on the positive results from the Comet assay (█████) in all tissues tested the concern that TGMDA may cause heritable changes to DNA is increased and this concern should be clarified without undue delay by exposing and analysing germ cells. 28 days of exposure and a sampling of sperm collected from the vas deferens/cauda epididymis after 49 days (mouse) or 70 days (rat) remain as an option as the 'gold standard' for germ cell mutagenesis and will not be modified in the updated TGR guideline. Sampling at this time point ensures that the sampled sperm cells were mitotically active (as stem cells) during exposure, which is likely to make them more sensitive to DNA damage than postmeiotic stages of spermatogenesis. The request to analyse male germ cells from the vas deferens/cauda epididymis collected at an additional sampling time of a minimum of 49 days (mouse), or 70 days (rat), after the end of treatment is upheld.

Proposals for amendment by other MSCAs

Proposals for amendment (PfA) were received from four other MSCAs, who argued that there already was enough evidence to obtain a harmonized classification for Muta. Cat. 2 according to CLP for TGMDA. All four MSCAs proposed to omit the testing of somatic tissues in the TGR assay. All four MSCAs supported the request for testing germ cells in the OECD TG 488 TGR assay due to the unclarified concern for germ cell mutagenicity.

One MSCA argued that it was their understanding that according to the experts in the GTTC working group, the latest results of their evaluation indicate that the second option (28+28 days) appears to give higher sensitivity for germ cell mutagenicity, as in this setup, germs cells are sampled, which have been exposed during the stem cell divisions, but also during the faster cell divisions as spermatogonids. In the 28+49/70 days setup, germ cells are sampled which have been exposed only during the stem cell stage. The MSCA therefore proposed to request the 28+28 days setup of the TGR, to obtain the highest sensitivity, based on the latest scientific knowledge, even though this deviates from the current OECD TG from 2013.

Another MSCA commented that in the comments on the draft decision you had proposed a testing strategy, comprising of a TGR study (OECD TG 488) with 28+3 days sampling and a conditional follow-up study with a 70 days post-exposure period in the event the 28+3 days assay is negative. The MSCA acknowledged that this earlier sampling time is much less sensitive than a 70 days post exposure sampling time, in relation to detecting germ cell mutagens and that they would normally support the approach proposed by the evaluating MSCA. However, the MSCA noted that according to the dossier (submitted 6 April 2017) it is stated that due to the positive Comet assay, a TGR study is ongoing although the dossier does not go into details of the study design. Given the comments, the MSCA assumed that it includes the shorter 3 day sampling rather than 70 days and questioned why this was not mentioned in the Registrant's comments nor acknowledged by the evaluating MSCA in the draft decision. The MSCA further enquired if the evaluating MSCA had discussed this with you.

The MSCA noted that it would appear that you have already embarked on step 1 of your own 2 stage approach and that if this was already initiated in April 2017 the results must be available in the near future. The MSCA therefore believed that a positive result in this (28+3 days) study would still permit a conclusion on germ cell mutagenicity, as it is a demonstration that the test substance has reached the gonad and caused detectable mutations. The MSCA noted that this information would be available much sooner than waiting for the requested study and that in the event of a negative result you had proposed to carry out the (28+49/70 days (mouse/rat) study as requested in the draft decision. The MSCA further noted that he may not agree that the strategy you proposed is the best approach, but the MSCA thought it should be acknowledged that there is testing underway, which will inform on the need for the requested study. The MSCA therefore proposed that due to animal welfare considerations, the evaluating MSCA should agree that in the event that the study initiated by you gives an unequivocal positive response and the appropriate classification and risk management measures are adopted, further testing for germ cell mutagenicity is not required. Where a negative or equivocal result is obtained, the second element of the strategy should be initiated without undue delay; the 28+70 days sampling time TGR in rats.

<u>Response to proposals for amendment by other MSCAs</u>

The evaluating MSCA agrees that the positive result in the recently conducted Comet assay (in vivo genotoxicity test) in combination with the positive result from the Ames test (in vitro mutagenicity assay) is sufficient to obtain a harmonized classification for mutagenicity in Category 2 according to CLP (Regulation (EC) No 1272/2008) Annex I, 3.5.2.2.

"The classification in Category 2 is based on:

— positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: — somatic cell mutagenicity tests in vivo, in mammals;

or

— other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays......."

The text in bold above indicates that this particular part of the CLP criteria for Muta. Cat. 2 is the most relevant in this case.

The evaluating MSCA therefore accepted the proposal to delete the request for testing of somatic tissues (liver, glandular stomach and duodenum) and only to request testing of male germ cells from the cauda epididymis after 28 days of exposure and a sampling time of 49 days (mouse) or 70 days (rat) post exposure.

In regards to the proposal to evaluate germ cell mutagenicity by employing the 28+28 days testing strategy the evaluating MSCA agreed with the argumentation of the MSCA that the 28+28 days testing strategy of sampling seminiferous tubules may be more

sensitive than 28+49/70 days (in mice/rats) due to the fact that spermatogonial cells divide faster than stem cells and that the possibility of mutations to occur during replication may therefore be higher because the spermatogonial cells are exposed when employing this testing strategy. However, it is the opinion of the evaluating MSCA that there may be cell type specific differences in sensitivity between stem cells and spermatogonial cells that are unclarified at this point. Furthermore, the evaluating MSCA had not had a chance to review the data on which the revision of the TG 488 is being based because the findings of the GTTC working group on the new 28+28 days testing strategy are not yet publically available and have not yet been incorporated into the OECD TG. Therefore, there is a chance, that ultimately, the scientific community would not accept the 28+28 days testing strategy as the optimal design for germ cell testing (i.e. the best test design to choose as the initial TGR test). This could also have complications with regard to e.g. mutual acceptance of data under non-EU regulations. For these reasons, and since the 28+28 days testing strategy has not been proposed by you, the evaluating MSCA rejected the PfA and decided to keep the original option of the 28+49/70 days strategy for germ cell testing as it is currently recommended in the test guideline.

Regarding the PfA concerning the testing which has already been initiated by you, the evaluating MSCA has been in contact with you, and you informed the evaluating MSCA of your plan to conduct a TGR assay. This assay, to the knowledge of the evaluating MSCA commenced in September 2017 and it initially employed a testing strategy of 28+3 days with a potential follow up of 28+70 days in rats. The evaluating MSCA has communicated to your that the MSCAs have to reach an agreement in MSC before the Registrant will be allowed to go ahead with follow up testing and that the evaluating MSCA, cannot be sure of what the final decision is going to be before a final agreement has been reached in the MSC. According to you the testing you are conducting, has been requested outside of the EU. The evaluating MSCA has not seen any documentation to support this claim.

You did not mention this test in your original comments to the draft decision and it was therefore not reflected in the RCOM.

The evaluating MSCA rejected the PfA from the other MSCA on the grounds that accepting an animal test with an inappropriate test design (28+ days) could set an unfortunate precedent, which would allow Registrants to decide on the testing strategy under substance evaluation, including the choice to decide on the sensitivity of the conducted assays. This would undermine the authority of ECHA and the MSC and could ultimately lead to unnecessary animal testing if the test strategy chosen by the Registrant is not adequate to clarify the concern raised by the evaluating MSCA under substance evaluation.

According to your comments, you will in case of a negative result in germ cells at 28+3 days then analyse germ cells using the 28+70 days testing strategy (rats). While acknowledging this, the evaluating MSCA points out that a negative result in germ cells using the 28+3 days testing strategy would not indeed be sufficient for clarifying the

concern. More specifically, this sampling time may give false negative results in germ cells because the most sensitive (mitotically active) cell population has not been adequately exposed to the test material, see e.g. (O'Brien et al 2016, Tox Sci 152:363-371).

If you report data showing that the 28+3 days testing strategy yielded an unequivocally positive result in germ cells, this would normally be considered sufficient for harmonised classification in Muta. Cat. 1B and no further testing (TGR 28+49/70 days) would be needed.

<u>Registrants' comments to proposals for amendment by other MSCAs</u>

You agreed with MSCAs that the results of studies currently available, the positive bacterial mutation test (Ames test; OECD 471) and the alkaline comet assay (OECD 489), are theoretically sufficient to allow Muta. Cat. 2 classification according to CLP.

You disagreed with MSCAs that the positive result obtained in the alkaline comet assay (OECD 489) confirms the concern regarding induction of mutagenic effects on germ cells. You reiterated your comments regarding the fact that based on the microscopic evaluation of tissues no test article induced effect to the function or architecture of the liver, stomach or duodenum was observed and that the increase in genotoxicity in duodenum samples was not dose responsive.

You further noted that in your opinion it is unlikely that the registered (parent substance) can induce mutagenic effects on germinal cells because bioanalysis was unable to confirm that the registered substance is able to pass from the gastrointestinal tract to the circulatory system.

You commented that you partially agreed with the comment from the three MSCAs regarding the request to sample germ cells from the vas deferens/cauda epididymis. However, you commented that there has been an on-going discussion of the OECD TG 488 since it was first issued in 2011 and that this discussion continues today in the GTTC working group. The first version of the TG 488 incorrectly listed only vas deferens as source of sperm for analysis at 28+70 days (rat). You noted that the vast majority of the mature sperm are in the cauda epididymis and therefore current scientific advice is to collect testes and cauda epididymis but not vas deferens due to the limited presence of sperm. You noted that the TG was revised in 2013 in large part to include cauda epididymis and to correct the mouse spermatogenesis cycle from 41 to 49 days.

In response to the comments regarding the work of the GTTC working group by one MSCA you noted that there are several relevant points of discussion on biology and evolving thoughts on guidance from the GTTC Germ cell advisory group. According to you there is a strong 3R's push to expand on the wording in OECD TG 488 that already permits analysis of the contents of seminiferous tubules in testes at 28+3 days as an "adequate" assessment of male germ cell mutagenicity. This approach would, in your view, be in the interest of animal welfare and would abolish the need to dose a separate group of animals only to analyze sperm in cauda at the extended time point 28+70 days.

In response to the enquiry by another MSCA you confirmed that you had initiated a TGR study (28+3 days) that is currently underway and that the in-life phase of the study has now been completed. You further stated that mutation analysis has not started at this time and that the complete results of the current study are expected to be available soon (February 2018).

In response to the enquiry by a MSCA you confirmed that the test design had been discussed with the evaluating MSCA but that it was only after comments were submitted on the draft decision and subsequent conversations took place with contract laboratories that you became aware of the discussion of the GTTC working group on Germ Cell Mutagenicity.

You stated that the TGR study (28+3 days) will analyse the cauda epididymis, glandular stomach, liver and duodenum. The testes will also be stored frozen and analysed if deemed necessary based on the results of the cauda epididymis samples. Initially, this TGR assay was conducted at doses up to 1000 mg/kg bw day. However, at day 10 of dosing significant effects were identified along with weight loss in the high dose group. The animals could not tolerate the highest dose (1000 mg/kg bw/d) and due to the rapid progression of the effects from ca. day 7 there was insufficient time available to reduce the dose to tolerable levels and home in on the MTD and therefore two additional low dose groups were subsequently added to the study. The TGR (28+3 days) study has been conducted at doses of 10, 20, 50, 100, 200 and 1000 mg/kg bw/day.

You stated that the testes samples, which are currently frozen will be analysed if deemed necessary based on the results of the cauda epididymis samples.

You commented that if ECHA decides that further animals should be used in an extended study run solely for the germ cell endpoint then it is proposed that analyzing sperm from cauda epididymis will be a more valid approach rather than sampling sperm originating from the vas deferens. You commented that it is not possible to analyze somatic tissue at the extended time-point (28+70 days) over the risk of losing mutants in rapidly dividing tissues.

You therefore proposed that the most robust scientific "hazard" based approach is to conduct a 28+3 days design for an optimized somatic and adequate germ cell analysis of testes. You further commented that it would be biologically meaningless to analyze sperm (vas or cauda) at 28+3 days because those mature sperm for the most part were past the point of DNA replication and cell division during dosing.

You agreed with the MSCA that if the testes samples yield a positive result this would permit a conclusion of germ cell mutagenicity. In the event of a negative result you also agreed to conduct a 28+70 days study specifically designed for germ cells sampling sperm from cauda epididymis. The complete results of the current study are expected to be available by February 2018.

In response to the PfAs regarding classification of TGMDA, you commented that the analysis of the somatic tissues samples (duodenum, stomach and liver) from your 28+3

days study will either i) confirm what we already know regarding mutagenicity in somatic cells or ii) provide opposing results to those observed in the Comet assay and, in your view, thereby indicate that the Comet assay is not valid for epoxy based substances and therefore it would be unwarranted to classify the substance as a Cat. 2 mutagen.

<u>Consideration of the Registrant's comments of the PfAs</u>

The evaluating MSCA notes that you agree that the positive result in the Comet assay in combination with the positive result from the Ames test is sufficient to obtain a harmonized Muta 2 classification. Regarding your comment pertaining to the result of the somatic tissues in the TGR initiated you, the evaluating MSCA notes that in case a negative result is obtained, the reliability of this result will have to be evaluated together with the positive result obtained in the Comet assay. The members of the Risk Assessment Committee will evaluate all data using weight of evidence to ascertain whether a Muta. Cat. 2 harmonised classification is warranted.

Regarding your comments pertaining to the results of the Comet assay in regards to the concern for germ cell mutagenicity see the response under 'Consideration of the Registrant(s) comments regarding the Comet assay (Bruce 2017)'.

Regarding your comments pertaining to the ongoing work from the GTTC group and the limited presence of sperm in the vas deferens, the evaluating MSCA accepted your proposal and as a result the request for sampling germ cells from vas deferens/cauda epididymis was changed to cauda epididymis only.

The evaluating MSCA noted your comment regarding sampling of seminiferous tubules in testes at 28+3 days as an "adequate" assessment of male germ cell mutagenicity. However, the evaluating MSCA has not seen any data which would show that this time point would be fit for purpose/sensitive enough. On the contrary, in your own original comments to the draft decision you provided results from a mathematical model from the GTTC group, which show that analysing germ cells from testes at 28+3 days is not a sensitive testing strategy.

Samples from seminiferous tubules in testes, consist of a mixed cell population with germ cell stages ranging from spermatogonia to spermatids, however, the majority of collected germ cells will be spermatids. Spermatids will mostly have been exposed in their post-mitotic stages when DNA synthesis (DNA replication and cell division) has ceased, which means that the time of exposure is not the most sensitive for fixation of mutations. According to the result of the mathematical model you provided in your original comments to the draft decision, spermatids will only have been exposed for an average of 1.3 days as stem cells and 8.4 days as spermatogonial cells. It is therefore likely that the large percentage of spermatids sampled from the testes could mask a mutagenic effect in the more sensitive cell populations, which again may result in a false negative result of the study.

In support of this, several studies in transgenic mice using acute exposure of known germ cell mutagens have shown that analyses of seminiferous tubules (or whole testis) 3

or 7 days after exposure did not show a significant effect, but that analyses at later time points yielded positive results (Hachiya et al (1999); Hoorn et al (1993); Katoh et al (1994); Hara et al (1999); Suzuki et al (1999)).

The data from the studies quoted above are based on mice. In rats the spermatogenesis has a longer duration (70 days versus 49 days in mice) and therefore the time point of 28+3 days will be even more insensitive in this species, (which you has used in your study).

As also stated by you, it is biologically meaningless to analyze sperm (vas or cauda) at 28+3 days because those mature sperm for the most part were past the point of DNA replication and cell division during dosing. Therefore mutations will not occur during these cell stages.

Your proposal to conduct a study at 28+3 days for somatic tissues and germ cells from the testes is therefore rejected. The concern for somatic mutagenicity has already been clarified by the Comet assay, which was requested in the ECHA decision adopted in 2015. The Comet assay is a validated OECD test guideline, and to the knowledge of the ecaluating MSCA there are no published records that this test should not be applicable to epoxy based substances.

The evaluating MSCA rejected the 2-step testing strategy that you had proposed and already initiated on the grounds that accepting an animal test with an inappropriate test design (28+3 days) could set an unfortunate precedent, which would allow Registrants to decide on the testing strategy under SEv, including the choice to decide on the sensitivity of the conducted assays. The request to test germ cells at 28 + 49/70 days (mouse/rat) is upheld.

However, the evaluating MSCA will carefully consider the different options with regard to enforcement / new draft decision if you later submit information that does not correspond to the requested test. In case you e.g. report data showing that the 28 + 3 days testing strategy yielded an unequivocally positive result in germ cells (either in cauda epididymis or testes), which is sufficient for harmonized classification in Muta. Cat. 1B, then the evaluating MSCA will not insist on unnecessary testing according to a TGR 28+49/70 days (mouse/rat) design. In case the result from germ cell testing is negative this will not be considered adequate to clarify the concern for germ cell mutagenicity.

The evaluating MSCA noted that you agreed to conduct a 28+70 days study specifically designed for germ cells sampling cauda epididymis in the event of a negative result in germ cells from testes in the 28+3 days study.

<u>Conclusion on the Comet assay OECD TG 489:</u>

The test material TGMDA UVCB induced statistically significant increases in DNA damage (DNA strand breaks) in all tissues tested (liver, stomach and duodenum) in Sprague-Dawley rats under the conditions of this study. The positive response was dose dependent in stomach and liver. The Comet assay has been conducted according to the

specifications requested in the decision on substance evaluation from 2015 and has been evaluated by the evaluating MSCA as reliable without restrictions, Klimisch 1.

Summary and conclusion on mutagenicity:

The results of the Comet assay from 2017 show that TGMDA induces genotoxicity *in vivo* in all tissues tested (liver, stomach and duodenum). The concern for gene mutations *in vivo* remains and is increased by this positive result. There is a concern for gene mutations in somatic cells and in germ cells *in vivo*. A follow up TGR OECD TG 488 test for gene mutations is required in order to clarify whether a proposal for a harmonized classification of Muta. Cat. 1B is relevant.

Furthermore, the Comet assay has a high sensitivity for predicting rodent carcinogens: A scientific review by Kirkland and Speit, (Mutation Research 654 (2008) 114-132), which assessed the sensitivity and specificity of UDS, TGR and Comet for rodent carcinogens showed that the Comet assay has a higher sensitivity than the TGR, but a similar specificity.

The concern for carcinogenicity is supported by positive QSAR predictions for monoconstituent TGMDA within the applicability domain of all of the carcinogenicity models from the Danish (Q)SAR database (http://qsardb.food.dtu.dk/database/index.html). Predictions were made for monoconstituent TGMDA in a commercial MultiCASE CASE Ultra FDA cancer suite consisting of seven models for cancer in male rat, female rat, male mouse, female mouse, rats, mice and rodents, respectively. Monoconstituent TGMDA was predicted positive in all of these cancer models.

These results indicate that TGMDA may be a genotoxic, non-threshold carcinogen.

Based on *in vitro* results from the Ames test OECD TG 471 it is likely that it is not the substance itself but an active metabolite of TGMDA that has the genotoxic effect.

Why new information is needed

Taking into account the tonnage (100 - 1,000 T/year) and the uses of the substance, including widespread uses by professional workers, a risk for human health cannot be excluded. The potential for germ cell mutagenicity and carcinogenicity of the substance needs to be clarified pursuant to Article 46(3) of the REACH Regulation, i.e. the immediate focus is to clarify whether the registered substance should get a harmonised classification for mutagenicity of the substance under Regulation (EC) No 1272/2008.

What is the possible regulatory outcome

At present TGMDA does not have a harmonised classification for mutagenicity. There is a concern that the substance and/or its metabolites are mutagenic in germ cells and/or somatic cells. If this is the case, there are no appropriate regulatory measures in place to ensure safe use. It is noted that a harmonized Muta. Cat. 1B classification in accordance with the CLP Regulation would elicit various downstream risk management measures according to existing EU legislation, which would limit the exposure to TGMDA and will also make it possible for an EU CA to propose to include TGMDA on the Candidate List of REACH as an initial step in the Authorisation REACH procedures.

Furthermore, if the analysis of germ cells from the cauda epididymis tissue in the OECD 488 TGR assay leads to a harmonized classification of Muta. Cat. 1B, the derived regulatory risk management measures from this classification would limit human exposure sufficiently so that the concern for carcinogenicity would not have to be clarified.

Considerations on the test method and testing strategy

The only suitable and available standard test method with which to assess gene mutations in germ cells is the TGR, OECD TG 488. According to OECD TG 488 the mouse or rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in mice or rats.

Germ cells from cauda epididymis are requested to be analysed in order to investigate germ cell mutagenicity.

The duration of exposure should be 28 days and sampling of cauda epididymis should be done 49 days (mouse)/70 days (rat) after the end of exposure.

ECHA considers that for mutagenicity testing the oral route is the most appropriate route of administration except for substances that are gases at room temperature. Hence ECHA concludes that testing should be performed by dissolving the registered substance in a suitable vehicle by the oral route (gavage).

As TGMDA is a reactive substance it may react in the administration formulation and hence a freshly prepared testing dose in an appropriate vehicle shall be used. A minimum of three, appropriately-spaced dose levels shall be used. To ensure a maximal exposure to unreacted TGMDA, preparations of test formulations shall be freshly made daily in the new study, no later than 20 minutes before administration of each dosage. Analyses of homogenicity and stability of the test formulations shall be performed. This shall be documented in the study report. The duration of the gavage procedure for each group shall also be documented in the study report.

The evaluating MSCA must have access to the full study report from the requested study including all relevant details of the study. Access to such detailed test report information is in the experience of the evaluating MSCA often needed to ensure that a clear conclusion regarding the result of the study can be drawn.

Considerations on the test material:

It is up to the Registrant(s) whether to conduct the study with the UVCB TGMDA or the monoconstituent TGMDA (see above).

Consideration of alternative approaches

The request for the OECD 488 is suitable and necessary to obtain information that will allow to clarify whether there is a risk for human health. More explicitly, there is no equally suitable alternative way available of obtaining this information. If the data, once obtained, confirms that the registered substance causes mutagenic effects, the authorities will consider further regulatory risk management measures such as harmonised classification and identification as SVHC. ECHA notes that there is no

experimental study available that will generate the necessary information without testing on vertebrate animals. Further, it was already stated in the ECHA decision sent to the Registrant(s) on 16 December 2015 that "In case the evaluating MSCA finds that the test result of the OECD 489 is positive, equivocal or that the test criteria are not acceptable the evaluating MSCA may then in a subsequent decision making process propose to request a TGR assay (Article 46(3) of the REACH Regulation)."

Conclusion

In conclusion, the concern of whether TGMDA and/or its metabolites cause gene mutations *in vivo* in germ cells remains. There is no alternative to obtain this information other than to conduct experimental studies, in particular there is no suitable *in vitro* method available.

Therefore, based on the substance evaluation and in accordance with Article 46(3) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the registered substance 4,4'-methylenebis[N,N-bis(2,3-epoxypropyl)aniline] (monoconstituent TGMDA, CAS 28768-32-2) or the UVCB TGMDA (CAS 28390-91-2) with the composition specified in the registration dossier subject to this decision:

Human health endpoint mutagenicity

Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays in mouse or rat by oral gavage (EU B.58./OECD 488) following a 28-day exposure with a subsequent 49 days (mouse), or 70 days (rat) sampling period. Male germ cells from the cauda epididymis shall be sampled and analysed.

Dosing shall be done by oral gavage daily in a freshly prepared test solution using an appropriate vehicle.

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All other references are included in the registration dossier

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to mutagenicity, carcinogenicity and wide dispersive use, 4,4'-methylenebis[N,N-bis(2,3-epoxypropyl)aniline], CAS No 28768-32-3 (EC No 249-204-3) (TGMDA) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013. The updated CoRAP was published on the ECHA website on 20 March 2013. The Competent Authority of Denmark was appointed to carry out the evaluation.

Based on the registration dossier and other available information the evaluating MSCA considered that further information was required to clarify the abovementioned concerns.

A unanimous agreement of the Member State Committee on the first draft decision was reached on 13 October 2015.

ECHA took the decision on 16 December 2015 pursuant to Article 51(6) of the REACH Regulation.

In that decision the Registrant(s) was required to submit the following information:

1.A Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays in mouse or rat by oral gavage (EU B.58./OECD 488) following a 28-day exposure with a subsequent 3 day sampling period. The following tissues shall be analysed: Glandular stomach, duodenum/jejenum, liver, and bone marrow. In accordance with paragraph 35 of the test guideline 'spermatozoa from the vas deferens/cauda epididymis and developing germ cells from the seminiferous tubules (as described in Paragraphs 32 and 33) should be collected and stored in case future analysis of germ cell mutagenicity is required.' If the analysis of any of the somatic tissues indicates that the substance is a somatic cell mutagen, the germ cell samples should shall then also be analysed.

OR

1.B In vivo mammalian alkaline Comet assay in mouse or rat by oral gavage (OECD 489) as a first in vivo test. The following tissues should be analysed (liver, glandular stomach and duodenum/jejunum). The optimum sampling time(s) should be determined based on kinetic data if available.

On 6 April 2017 the Registrant(s) submitted an updated registration dossier, which included the result of an *In vivo* mammalian alkaline Comet assay in rat by oral gavage (OECD 489).

Based on the updated registration dossier and other available information the evaluating MSCA considered that further information was required to clarify the concern from germ cell mutagenicity. Therefore, it prepared a second draft decision pursuant to Article 46(3) of the REACH Regulation to request further information. It submitted the second draft decision to ECHA on 4 July 2017.

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

The decision making followed the procedure of Articles 50 and 52 of the REACH

Regulation as described below.

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took into account your comments which were sent within the commenting period, and they are reflected in the reasons (Appendix 1). The request(s) were amended.

Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision and modified the draft decision. They are reflected in the reasons (Appendix 1). The request to analyse somatic tissues (glandular stomach, liver and duodenum) in the requested OECD TG 488 were withdrawn.

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendment(s).

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

MSC agreement seeking stage

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-57 meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

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

2. Failure to comply with the request(s) in this decision, or to otherwise fulfil the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used ('test material') has to have a composition that is within the specifications of the substance composition that are given by all registrant(s). It is the responsibility of all the registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on the 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.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who will 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 will designate one of the registrants to perform the stud(y/ies) on behalf of all of them.