ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 19 May 2017

Registered substance name: reaction product: bisphenol-A-(epichlorhydrin); epoxy resin (number average molecular weight ≤700)
EC No: 500-033-5
CAS No: 25068-38-6
Date of Latest submission(s) considered[1]: December 2016
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)
Addressees: Registrant(s)[2] of the substance: reaction product: bisphenol-A- (epichlorhydrin); epoxy resin (number average molecular weight ≤ 700) (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on **the main constituent of the registered substance, 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxy-methylene)] bisoxirane, BADGE,** EC No 216-823-5; CAS No 1675-54-3:

1.1 Human health endpoint mutagenicity

1.1 A Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays (TGR) 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 and liver. 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 of the test guideline) shall 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 shall then also be analysed.

OR

1.1 B In vivo mammalian alkaline comet assay; test method: OECD 489 in rats, oral route (gavage), in the following tissues: liver, glandular stomach and duodenum. The optimum sampling time(s) should be determined based on relevant kinetic data if available, but otherwise the default sampling times in OECD 489 should be employed. Two sets of slides shall be prepared and analysed, one set submitted to standard experimental conditions and one set submitted to modified experimental conditions that enable the detection of DNA-DNA and DNA-protein crosslinks (according to e.g.

[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.

references 23, 36-39 in the TG 489 or Pant et al. 2015). A specific positive control for DNA cross linking effects shall be included.

The full study report from the requested study including all relevant details of the study must be made available to the evaluating Member State Competent Authority (evaluating MSCA).

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on **the registered substance** (EC No 500-033-5, CAS No 25068-38-6):

1.2 **CSR-related requests**

1.2.1 Revision of Section 5.11 in the CSR on calculation of overall assessment factors (AF) in the derivation of DNELs using ECHA guidance recommendations (ECHA's *Guidance on information requirements and chemical safety assessment Volume 8, Chapter R.8*), or including a substance specific justification for using other AFs, as further specified in Appendix 1.

1.2.2 Further specifications to workers on the use of personal protective equipment as further specified in Appendix 1.

You shall provide an update of the registration dossier(s) containing the requested information, including robust study summaries and an update of the Chemical Safety Report by:

- **27 May 2019** - 24 months from the date of the decision, should you perform the requested test under option 1.1 A for mutagenicity

or

- **26 November 2018** - 18 months from the date of the decision, should you perform the requested test under option 1.1 B for mutagenicity.

The respective deadlines take into account the time that you may need to agree on who is to perform any required tests.

The reasons of this decision 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.

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2. Appeal

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

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

[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 on "reaction product: bisphenol-A-(epichlorhydrin); epoxy resin (number average molecular weight ≤ 700)" (CoRAP name); in the registration dossiers also known as 4,4'-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane and abbreviated DGEBA in the following, and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating MSCA to complete the evaluation of whether the substance constitutes a risk to human health and/or the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested in order to clarify the concerns for the identity of the substances tested, for mutagenicity and for human exposure.

Tiered/Conditional testing strategy: The information requested above under point 1.1: Human health endpoint mutagenicity constitutes the first tier in a testing strategy to clarify the concerns for gene mutations *in vivo*. Hence, the evaluating MSCA will review the information submitted by the Registrant(s) as an outcome of tier 1 of the testing strategy, and evaluate if further information should be requested in order to clarify the concern for somatic and germ cell gene mutagenicity.

Note on the identity of the substance

ECHA notes that the substance is registered as a mono-constituent. However, it appears among other things that content of the main constituent, 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxy-methylene)]bisoxirane, BADGE (EC No 216-823-5; CAS No [redacted]) may not be in line with the ECHA requirements for a mono-constituent substance, as the ECHA guidance refers to a normal minimum content of 80% of the main component in a mono-constituent substance. In order to clarify the identity of the registered substance, ECHA has launched a targeted compliance check with respect to substance identification.

Due to the serious nature of the concerns for human health (potential mutagenicity via a non-threshold effect) identified in the substance evaluation, it was decided to proceed with the substance evaluation in parallel to the compliance check evaluation.

The testing requirement of this decision is related only to the main constituent, BADGE, of the registered substance. Therefore, the compliance check on the substance identity of the registered substance does not influence the present decision. However, clarification on substance identity may have consequences for the further substance evaluation of the registered substance in the future.

The decision from the compliance check on substance identity was sent to you on 14 December 2016, and the deadline for you to submit the required information was 21 March 2017. However, as the result of the compliance check process is not expected to impede on the testing requirement of the present decision, the substance evaluation process continues in parallel.

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ENDPOINT 1.1: Human health end-point Mutagenicity

The Concern(s) Identified

The concern for mutagenicity included in the justification for CoRAP listing of the registered substance (DGEBA) is that the substance and/or its main constituent BADGE (CAS no 1675-54-3) has been tested positive in several *in vitro* mutagenicity tests investigating gene mutations and chromosomal aberrations. The available follow up tests for *in vivo* mutagenicity address only chromosomal aberrations and consequently were unable to address the remaining concerns regarding the ability of the substance and/or its reactive metabolites to induce gene mutations in somatic and germ cell tissues.

A review of the available studies on mutagenicity has been performed under this substance evaluation. Unless otherwise stated all evaluated studies were part of the registration dossier.

***In vitro* studies**

Gene mutations in bacteria and yeast

Several studies have confirmed that DGEBA and BADGE yield a positive result in the AMES test in base-pair substitution strains of *Salmonella* TA100 and TA1535. BADGE/DGEBA causes gene mutations in TA100 and TA1535 with a dose-related increase both with and without metabolic activation. Two studies (both reliable (evaluating MSCA: Klimisch score 2)) including one found in the open literature have shown that BADGE caused gene mutations in the *E. Coli* strains WP2*uvrA* and IC3327 (Sueiro *et al.,* 2006), indicating that BADGE may cause oxidation or cross-linking of DNA.

Gene mutations in mammalian cells

Several thymidine kinase (TK) mouse lymphoma forward mutation assays (evaluating MSCA: Reliable (Klimisch score 2)) have yielded positive results for DGEBA (positive with and without metabolic activation). Forward mutations in the TK gene can be caused by both gene mutations or chromosomal aberrations. Slow growing cells are the result of chromosomal aberrations. However, it was not possible to assess the potential for chromosomal aberrations in the studies due to the incubation time of the cells being too short.

Gene mutations at the HPRT locus were also investigated (V79 cells). Test materials were BADGE (>98 % purity) and BADGE-bis-diol (>98 % purity), which is the primary metabolite of the registered substance in animals. Results without metabolic activation showed that the mutation rate was elevated at 10µM and 15 µM for BADGE, but due to high variation this was not statistically significant. A significant elevation in mutation frequency was seen at 20µM for BADGE (plating efficiency was 4-36 %, so the positive result could at least partly be due to cytotoxicity). The hydrolysis product BADGE-bis-diol did not elevate the mutation rate compared to controls. BADGE and BADGE-bis-diol were also tested with metabolic activation (in an FSB free medium). Levels 50 µM and 100µM did not induce mutagenicity nor cytotoxicity.

Chromosomal aberrations *in vitro*

A positive result was seen in an *in vitro* mammalian chromosome aberration test similar to OECD 473, which used monolayer slide culture of rat liver with endogenous metabolic capacity. There was a dose-related increase in the frequency of chromatid gaps, chromatid breaks, acentric fragments and chromatid exchange figures in cultured RL4 cells exposed to DGEBA

and BADGE. There was also an increase in the frequency of chromatid gaps, chromatid breaks and chromatid exchange figures in cultured RL1 cells exposed to BADGE at 15 µg/ml (evaluating MSCA: Reliable (Klimisch score 2)).

Micronucleus test *in vitro*

In one study BADGE and the hydrolysis product BADGE-bis-diol were tested in a micronucleus assay similar to OECD 487 (V79 cells) with and without metabolic activation. Antikinetochore antibodies (CREST) were used to characterize the induced micronuclei. BADGE without metabolic activation induces CREST negative micronuclei at 50 µM, which consisted of acentric chromosomal fragments and did not contain whole chromosomes/chromatids, which indicates that BADGE is clastogenic, but not aneuploidogenic. BADGE with metabolic activation did not induce micronuclei. In the presence of metabolic activation cells displayed changes in morphology and growth inhibition was reported for up to 10 hours after treatment. These effects are not further described in the study. A post treatment time of 3-6 hours is required in order to assess the potential for CREST positive micronuclei. Because of the cytotoxic effects the aneugenic potential of BADGE with metabolic activation could not be assessed. BADGE-bis-diol did not induce micronuclei (evaluating MSCA: Reliable (Klimisch score 2)).

A micronucleus study with three experiments was performed with human peripheral blood lymphocytes from two donors *in vitro* with and without metabolic activation with BADGE and, BADGE hydrolysis products BADGE-mono-diol and BADGE-bis-diol. All test materials statistically increased cells with micronuclei without metabolic activation in a dose-dependent manner. BADGE also statistically increased cells with micronuclei with metabolic activation in a dose-dependent manner, but the hydrolysis products did not. There was an increase in micronuclei at the highest dose level for one of the two donors - with metabolic activation.

DNA adduct formation

DGEBA has been shown to react with the nucleoside deoxyguanosine in an alkylation assay, making it likely that it would form DNA adducts. Higher molecular weight bisphenol A derivatives Epikote 1001 and 1004 were also tested. DNA adduct formation decreased with an increase in molecular weight.

***In vitro* mammalian cell transformation assay**

Dose related increases in transformation frequency were observed for both DGEBA and BADGE in the *in vitro* mammalian cell transformation assay (genome mutation) using Syrian hamster fibroblast kidney cells (BHK 21/Cl13) with metabolic activation. (evaluating MSCA: Reliable (Klimisch score 2)).

In vivo studies in somatic cells

Chromosomal aberrations

Three micronucleus assays (structural or numerical chromosomal aberrations) similar to the OECD 474 mammalian erythrocyte micronucleus test have been conducted in rodents and have all yielded negative results: A micronucleus assay (chromosome aberration) was conducted in male/female mouse by oral gavage with BADGE. BADGE in corn oil was tested at 0, 500, 2500, 5000 mg/kg (nominal conc.). Cyclophosphamide (80 mg/kg/day) was used as a positive control. Five males and 5 females were assigned to each group. Animals were killed 24, 48 and 72 hours after dosing. The result was negative: BADGE did not significantly increase micronuclei in bone marrow polychromatic erythrocytes (PCE) under the testing

conditions. 1000 PCEs were scored per animal. The PCE:NCE -cell ratio was not reduced by the positive control. There was a reduction at some dose levels for BADGE, but it was not consistent and at some dose levels the ratio was increased in DGEBA groups compared to the negative control (evaluating MSCA: Reliable (Klimisch score 2)).

A chromosome aberration study in bone marrow cells of Chinese male/female hamster was conducted where DGEBA in PEG was given by oral gavage on 2 consecutive days to groups of 6 male and 6 female hamsters at 825, 1,650 and 3,300 mg/kg bw/day. Animals were killed 24 hours after the last treatment. Cyclophosphamide was used as a positive control. The result was negative: DGEBA did not significantly increase chromosomal aberrations in bone marrow cells under the testing conditions. 1000 cells were scored per animal. The test was not an OECD guideline study (evaluating MSCA: Reliable (Klimisch score 2)).

Another chromosome aberration study (micronucleus test in bone marrow of Chinese hamsters (male/female)) was conducted. Sample size was 2 male + 2 female per group. Hamsters were exposed by oral gavage on 2 consecutive days to 0, 825, 1650, 3300 mg/kg to DGEBA. Hamsters were sacrificed 6 hours after the second administration. The positive control substance used was cyclophosphamide. Bone marrow chromatid- and chromosome-type aberrations were examined following oral administration of test material to hamsters. DGEBA did not significantly increase chromosomal aberrations in bone marrow cells. 1000 "metaphase plates" were scored per animal. The test was not an OECD guideline study. The group size was very small (evaluating MSCA: Not reliable (Klimisch score 3)).

Assessment of single strand breaks by an alkaline filter elution assay

Genotoxicity *in vivo* of BADGE was investigated with an alkaline filter elution assay, which assesses single strand breaks (SSB) and alkaline labile sites in DNA. Prior to experimentation a partial hepatectomy was performed on Wistar rats and at the peak of the restorative DNA synthesis induced by the surgery liver DNA was labelled with radioactive thymidine isotopes. BADGE as a 20 % solution in DMSO was administered to rats via oral gavage 6 hours after one dose of 500 mg/kg, and methyl methanesulphonate was administered in DMSO as a positive control. Cells are layered onto a PVC membrane and washed with cold phosphate-buffered saline (PBS) and a lysing solution. Single strand damage is assessed as a reduction in single strand molecular weight (observed as an increase in rate of elution of radioactivity going through the filter). The rate of elution depends on the length of the single strands. No increase in SSBs was observed in the BADGE group. The positive control yielded a reduction of more than 30 % at the end of the experiment. The assay only assesses SSB because single strands are able to pass through the filter whereas BADGE covalently bound to DNA strands would not. This is not a guideline study and only one dose was tested. No protease was used in the lysing solution, so it is possible that single strand breaks could still be adducted to proteins, which would mask a positive result (evaluating MSCA Klimisch score 3, not reliable).

DNA adducts *in vivo*

Covalent binding to DNA has been detected in mouse skin after topical application of BADGE. The DNA adducts formed were identical to those formed by the BADGE metabolite glycidaldehyde. Glycidaldehyde has been shown to be carcinogenic in mouse skin. The alkylation frequency was 0.1-0.8 adducts/106 nucleotides following dosing with 2 mg BADGE per mouse and 166 adducts/106 nucleotides after 2 mg glycidaldehyde per mouse. Thus, the amount of DNA adducts after application of BADGE was several orders of magnitude lower than that observed after application of glycidaldehyde.

***In vivo studies in* germ cells/offspring**

Chromosomal aberrations test in mouse spermatocytes

A chromosome aberration study similar to OECD 483 was conducted in male germinal epithelium in mouse (NMRI) by oral gavage. DGEBA in PEG 400 was given 5 times over a period of 10 days (day 0,2,3,5 and 9) at doses of 1000 mg/kg, 3000 mg/kg and 10,000 mg/kg (nominal conc.) bw/day (8 male mice per group). All animals died in the 10,000 mg/kg group. Seven of 18 died in the 3000 mg/kg group and 2 of 15 died in the 1000 mg/kg group. Animals were killed 3 days after the final dose (3 hours after an i.p. injection of colcemide). Then 100 metaphases each of primary and secondary spermatocytes were examined from each animal. BADGE did not induce an increase in chromosomal aberrations study in male germinal epithelium in this study. Results showed 3 aberrations (1 primary, 2 secondary) of 1600 metaphases total in the control group. For the 1000 mg/kg group fragments included 2 aberrations in the primary spermatocytes and 1 aberration in the secondary spermatocytes. For the 3000 mg/kg group fragments included 3 in the primary spermatocytes and 1 in the secondary spermatocytes. Health Council of the Netherlands 2013 and EFSA 2004 refer to this study as being inconclusive due to an exposure period of only 5 days: "The time interval between the last DNA synthesis and first meiotic division in mouse male germ cells is 11 days:, therefore because most chemical clastogens are S-phase dependant, sampling for cytogenetic analysis of spermatocytes should have been done 11 or more days after treatment instead of 8". However, sampling in this study was in fact done 13 days after the first treatment. Very high doses were used in this study and the duration of exposure lasted 10 days. However, no positive control was used (evaluating MSCA: Klimisch score 2-3).

Another chromosome aberration study in male germinal epithelium study in NMRI mice was conducted in 1984 using DGEBA: Dosages used were 375, 750, 1500 and 3000 mg/kg bw/day given once a day for five consecutive days. Mice were killed 24 hours after the final dose (3 hours after an i.p. injection of colcemide). Testes from 16 animals in each of the treated groups and 22 animals in the control groups were processed. Then 100 metaphase figures from each of 8 animals in each control group were scored for aberrations: No specific aberrations were found in the control group and in the 375 mg/kg group. In the 750 mg/kg group 1 chromatid exchange was found. In the 1500 mg/kg group 1 metaphase with 2 chromatid breaks was seen and in the 3000 mg/kg group 1 metaphase with a chromatid fragment was found. No dose-related increase in the frequency of aberrations was seen. The result was equivocal. No positive control was used in this study (evaluating MSCA: Not reliable, Klimisch score 3).

Dominant lethal assays

A dominant lethal assay using oral gavage (single dose of either 3,333 mg/kg bw/day or 10,000 mg/kg bw/day). Twenty male albino mice (Tif: MAG f (SPF)) per group, were mated to untreated females from the same strain over a period of 6 weeks. Females were necropsied on the 14th day of gestation. Number of live embryos, embryonic deaths and sites of early embryonic resorptions were counted. There was no difference between DGEBA groups and vehicle controls. No positive control was used in this study.

Another dominant lethal assay was conducted with test material applied dermally at 3000 mg/kg bw/day to male mice (topically, undiluted). Ten males per group were treated 3 times per week for a minimum of 8 weeks. Females were killed 13-14 day after presumed mating. There was no significant increase in the DGEBA treated groups compared to the control group in the number of live embryos, embryonic deaths or sites of early embryonic resorptions.

However, the proportion deaths/pregnancy for this compound was consistently and significantly lower than the controls. Individual data points were not available in the report. The total number of pregnancies in each group was also not available. Triethylenemelamine was used as a positive control.
The endpoint of the dominant lethal assay (lethal chromosomal aberrations) is not very sensitive and it is the opinion of the evaluating MSCA that these test results cannot clarify the concern for chromosomal aberrations in germ cells.

Summary for the concern of mutagenicity

DGEBA has yielded positive results for gene mutations *in vitro* in the Ames test both with and without metabolic activation (evaluating MSCA: Reliable (Klimisch score 2)). Positive results in the *E. Coli* strains WP2uvrA and IC3327 from the Ames test indicate that DGEBA could be a cross-linking or oxidizing mutagen. DGEBA has also been shown to bind to DNA both *in vitro* and *in vivo*. Even though the order of magnitude of DNA binding *in vivo* was very low this along with the result from the Ames test indicates that DGEBA could be a cross-linking mutagen. Positive results for gene mutations were also observed in mammalian cells *in vitro* without metabolic activation. Metabolic activation was only investigated in one of these studies and yielded an equivocal result.

Positive results were observed for chromosomal aberrations *in vitro* in a mammalian chromosome aberration test using cultures of rat liver and in two micronucleus tests without metabolic activation.

The two reliable (evaluating MSCA: Klimisch score 2) *in vivo* studies, which investigated chromosomal aberrations in somatic cells (bone marrow) yielded negative results. Two other studies addressing chromosomal aberrations in mouse spermatocytes yielded negative/equivocal results and although the results of these studies are inconclusive due to the inadequate study protocols used, in one study a high dosage was used for 10 days duration, which indicates that DGEBA is not likely to cause chromosomal aberrations under these study conditions. Based on all the available data regarding chromosomal aberrations *in vivo* there is no remaining concern for structural or numerical chromosomal aberrations in distant tissues such as the bone marrow and testes. However, there is a remaining concern for genotoxicity in initial site of contact tissues *in vivo*: DGEBA has namely yielded positive results in an *in vitro* alkylation assay and BADGE has also shown covalent binding to DNA in mouse skin *in vivo*. Genotoxicity (strand breaks) has been investigated *in vivo* in the alkaline elution assay, but this study is not reliable (Klimisch score 3). There is a concern as to whether the registered substance and/or its reactive metabolites can cause genotoxicity *in vivo*. Furthermore, DGEBA and BADGE yielded positive results for bacterial and mammalian gene mutations *in vitro*. No studies investigating gene mutagenicity *in vivo* are currently available and there is therefore concern as to whether the registered substance and/or its reactive metabolites can cause gene mutagenicity in somatic and/or germ cells *in vivo*.

Why new information is needed

There is a potential risk of human health effects due to the mutagenic properties of DGEBA. The available genotoxicity data is unable to address the remaining concerns about the potential of DGEBA and/or its reactive metabolites to induce heritable gene mutations in germ cells (i.e. resulting to Muta Cat 1B classification according to the CLP Regulation), which would elicit various EU regulatory risk management measures to exclude or limit exposure to various

human populations / exposure scenarios.

In order to draw a robust conclusion on gene mutagenicity *in vivo*, a gene mutagenicity test such as the transgenic rodent (TGR) assay (OECD 488) may be needed. If this test shows that gene mutations are induced in somatic cells then germ cells should also be investigated in order to rule out or confirm heritable gene mutations (possible classification as Muta 1B).

However, if a reliable Comet assay (OECD 489) (one set of slides under standard conditions and one set of slides modified to enable the detection of DNA-DNA and DNA-protein crosslinks) with adequate and suitable sampling times and specific positive controls is performed instead and if this test yields a negative result for *in vivo* genotoxicity, this will give a strong indication that DGEBA would also yield a negative result for *in vivo* gene mutations.

If on the other hand the Comet assay yields a positive result, this would be followed up by considering a request for gene mutagenicity testing in both somatic and germ cell tissues in order to investigate whether a permanent change in the DNA takes place (mutagenicity). In case a positive result is obtained in a follow up TGR assay it would be possible to conclude on the appropriate classification for mutagenicity, which would in turn lead to downstream risk reduction measures, depending on the classification category (positive results in only somatic cells would lead to a Muta Cat. 2 classification whereas positive results in both somatic and germ cells would lead to a Muta Cat. 1B classification).

At present DGEBA 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, the regulatory measures in place to-day are not appropriate to ensure safe use. It is noted that a harmonised 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 DGEBA and also make it possible for an EU Competent Authority to propose to include DGEBA on the Candidate List of REACH as an initial step in the Authorisation REACH procedures.

Considerations on the test method and testing strategy on mutagenicity

Testing strategy

The TGR assay (OECD 488) listed as option 1.1 A under the end-point mutagenicity is able to detect gene mutations in somatic cells and in germ cells and is therefore suitable for clarifying the concern for gene mutations caused by DGEBA/BADGE. Option 1.1 B under the end-point mutagenicity, the Comet assay (OECD 489) is able to detect genotoxicity (strand breaks), which may be the result of either gene mutations or chromosomal aberrations and it is therefore a suitable and sensitive *in vivo* test for investigating genotoxicity in various somatic tissues in order to address the concern for gene mutagenicity. However, the OECD 489 has not yet been validated for detecting germ cell mutagenicity, so in case of a positive result in the Comet assay additional mutagenicity testing may be needed. A result from the Ames test showed that BADGE caused gene mutations in the E. Coli strains WP2uvrA and IC3327, which indicates that BADGE may cause oxidation or cross-linking of DNA. If BADGE causes cross-linking then a Comet assay with standard experimental conditions may yield a false negative result. The Comet guideline (OECD TG 489) states that DNA-DNA and DNA-protein crosslinks can be detected by the Comet assay under certain modified experimental conditions. You are

therefore requested to prepare and analyse two sets of slides when performing the Comet assay, one set to be submitted to standard experimental conditions and the other set submitted to modified experimental conditions that enable the detection of DNA crosslinks according to a reliable protocol, e.g.as described in the references 23, 36-39 of the TG 489 or Pant et al. (2015). A specific positive control for DNA cross linking effects shall be included.

The full study report from the requested study performed must be made available to the evaluating MSCA. The evaluating MSCA will evaluate and interpret the results of the test chosen to be performed and conducted according to the specifications in this decision and the test guideline. Should you choose to perform the test described under point 1.1 B, the evaluating MSCA will evaluate and interpret the results of the performed OECD 489 when available including the documentation and fulfilment of the acceptability criteria of the test guideline (paragraph 58-65 OECD 489) and the modifications and specific positive control to detect cross linking. In case the evaluating MSCA finds that the result of the test is clearly negative, it will then be concluded that there is no further concern for *in vivo* gene mutagenicity.

In case you decide to perform the OECD 489 and 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 propose in a new decision further testing, including the possibility to request a TGR conducted according to OECD 488 in appropriate tissues to be decided later. This may be necessary in order to be able to conclude on the concerns for somatic and germ cell gene mutagenicity.

Test specifications for the requested option 1.1 A (OECD 488) or option 1.1 B (OECD 489)

Test substance

The test should be conducted with the main component of the registered substance DGEBA, namely 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bisoxirane, BADGE: EC 216-823-5; CAS 1675-54-3. DGEBA is registered as a mono-constituent, and BADGE should therefore normally be present in an amount of at least 80% of the registered substance. Other components and impurities may vary in identity and concentration. Therefore, BADGE would represent the main component that has been tested in every test included under the end-point of mutagenicity, irrespective of whether the test substance was claimed to be DGEBA or BADGE, and regardless of the purity of the tested substance/material in the respective tests. BADGE is the component with the lowest molecular weight of the oligomer reaction products in registered substance (DGEBA). The reactive chemical group of concern with respect to mutagenicity, the epoxy-group, is present in the largest proportion in BADGE compared to larger molecular weight constituents of DGEBA. The positive results of the various mutagenicity tests most probably reflect hazardous properties of BADGE rather than of components present in low concentration or of impurities. Therefore, the concern for a possible mutagenic effect of DGEBA would mainly be due to BADGE.

ECHA issued a targeted compliance check decision on the substance identity of the registered substance. The outcome of this compliance check may influence the information in the registration dossier as regards the composition of the substance covered by the registration. However, it is unlikely that the identity of the main component will be changed. The primary concern for mutagenicity is linked to this component. Therefore, the above mentioned testing is required on the main component BADGE.

Administration route

DGEBA may expose humans via all administration routes. Exposure via dermal and oral routes are evident from the use profile of the substance and its intrinsic fate related properties. However, also inhalatory exposure may occur even though the DGEBA constituents have low vapour pressure because DGEBA is also registered for spray applications (both in industrial settings and non-industrial settings), i.e. aerosols may be generated and may subsequently be inhaled. Furthermore, use of the substance at elevated temperature may generate vapours which may also be inhaled. The main concern for genotoxicity/mutagenicity of the DGEBA constituents are related to the reactive epoxy groups which may directly react with nucleophilic macromolecules in the cells, i.e. more specifically with nucleophilic sites in certain amino acids of proteins and the bases of the DNA. Hence, direct site of contact genotoxicity/mutagenicity is a major concern even though genotoxicity/gene mutagenicity in more distant tissues cannot currently be excluded. All exposure routes are relevant. There is no information, which indicates that occurrence of possible genotoxic/mutagenic effects, including effects in initial site of contact tissues, may be dependent on the exposure route. Hence the oral route of exposure is chosen for this study (default administration route for genotoxicity/mutagenicity studies). As the reactive epoxy groups of the constituents of DGEBA may interact with feeding material, administration by gavage is selected.

Further specification regarding dosing

Preparation of test formulations shall be done with an appropriate vehicle, which shall be justified in the study report. As BADGE is a reactive substance it may react in the administration formulation. To ensure a maximal exposure to unreacted BADGE, preparations of test formulations shall be freshly made daily, shortly before each administration of the dosage. Analyses of homogeneity and stability of the test formulations shall be performed every week in order to ensure that the animals are exposed to unreacted BADGE.

Tissue selection and sampling time schedule

The liver is selected because the liver is the main metabolizing tissue whereas glandular stomach and the duodenum are requested because they are both considered initial site of contact tissues and it is currently not known whether or not inclusion of both tissues is required for a definite identification of initial site of contact mutagenicity. Hence to assure a definite identification both are required.

Test specifications for the requested option 1.1 B Comet assay (OECD 489)

Due to the transient nature of strand breaks the optimum sampling time is critical. The optimum sampling time(s) may be substance- and/or administration route-specific, sampling times should be determined where available from kinetic data (e.g. the time (Tmax) at which the peak plasma or tissue concentration (Cmax) is achieved, or at the steady state for multiple administrations). In order to maximize the ability to detect short-lived lesions animals should be euthanized and tissues collected at, or soon after Tmax is reached. In the absence of kinetic data a suitable compromise for the measurement of genotoxicity is to sample at 2-6 h after the last treatment for two or more treatments, or at both 2-6 and 16-26 h after a single administration, although care should be taken to necropsy all animals at the same time after the last (or only) dose. Information on the appearance of toxic effects in target organs (if available) may also be used to select appropriate sampling times. In addition, in order to determine the optimum sampling time for this study you shall examine the available data on

toxicokinetics from the registration dossier in detail. Furthermore, a search of the open literature must be conducted in order to retrieve any additional relevant and reliable information. After completing a review of the available information you shall then update the toxicokinetics section in the registration dossier in order to scientifically justify the chosen sampling time(s). If no relevant toxicokinetic information is available the default times in OECD 489 shall be used unless there is information available which indicate that certain deviations from that tissue sampling time may be scientifically more appropriate despite absence of relevant toxicokinetic data.

A concern for cross-linking is indicated by *in vitro* and *in vivo* studies for BADGE/DGEBA. Therefore, a modified Comet assay for detecting cross linking by inducing additional DNA damage such as treatment by methyl methanesulfonate (MMS) or ionising radiation (gamma or X-ray) according to a reliable protocol as described in the references 36-39 of the TG 489 (version 2016) and in Pant et al. (2015) is requested to be conducted alongside the Comet assay with standard experimental conditions in order to be able to clarify the concern for cross linking. A specific positive control (e.g. hexamethyl phosphoramide or cisplatine) shall be included in order to assess the reliability of the results.

All critical parameters described in the OECD 489 should be carefully controlled and documented in detail in the study report. You should consider also including examining gonadal cells, as it would optimise the use of animals (also refer to Appendix 3: Further information, observations and technical guidance for details).

The evaluating 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 the need to evaluate all study details relevant for the result because such details are based on experience not always available in robust study summaries only.

Further testing may later be relevant in accordance with Article 46 of the REACH Regulation, depending on the results obtained in the studies requested above.

Alternative approaches and Proportionality of the request

The substance has widespread use and is manufactured and imported in the EU in vast amounts (100,000-1,000,000 tonnes per annum). The concern of whether the substance and/or its metabolites cause gene mutations *in vivo* in germ cells and/or somatic cells remains. There is no alternative to obtain this information other than to conduct an experimental study, in particular as there is no relevant existing *in vivo* data or in vitro method available.

3R considerations

Both the Comet Assay and the TGR Assay utilize a total of ≥ 25 animals per test. This means that if a TGR Assay is chosen only around 25 animals will be utilized in total because the concern for somatic and germ cell mutagenicity can be resolved in the same animals in accordance with two of the 3R principles of reduction and refinement.

If the option of conducting a Comet Assay as a first *in vivo* test is chosen, ≥ 25 animals will be used for this test. If the Comet Assay yields a positive result and is followed up by a TGR assay, ≥ 50 animals will be utilized in total. It is noted that there is no experimental method

available at this stage that will generate the necessary information which does not employ vertebrate animals.

The request for the Comet assay OECD 489 is hence both suitable and necessary to obtain information that will allow clarification of whether there is a risk for gene mutations *in vivo*. More explicitly, between different available alternatives, the Comet assay is the least onerous way to obtain relevant information (providing that the Comet assay yield a negative result). If, however, the data from the Comet assay once obtained indicate that the registered substance causes genotoxicity in somatic cells, this will indicate that there may be a risk of gene mutations in somatic and perhaps even in germ cells *in vivo*. A follow up with suitable further information requirements including the possibility to request a suitable *in vivo* gene mutagenicity test, i.e. the TGR assay, will then be required in order to obtain results that may indicate whether a proposal for a harmonized classification of Muta Cat. 2 or 1B would be relevant. If such a harmonized classification were to be concluded, this would lead to further regulatory risk management measures in various EU downstream regulations. In particular if MUTA 1B is concluded this will lead to a range of downstream regulatory measures limiting human exposure to DGEBA as well as raising the possibility to nominate the substance for the Candidate List and ultimately to the authorisation scheme of REACH.

Consideration of registrants' comments on the draft decision, of Member States' proposals for amendment (PfAs) and of registrants' comments to the PfAs:

You commented to the initial draft decision on the deadline of 15 months given, arguing for an extension to 18-24 months. The deadline was, however, initially not changed on the basis of those comments.

You committed in your comments to the initial draft decision to perform the standard Comet assay (OECD 489) on the main constituent of the registered substance: BADGE requested at that time.

A proposal for amendment (PfA) from one Member State proposed to request duodenum in the Comet assay and not duodenum/jejunum. The evaluating MSCA accepted this PfA. You agreed to this PfA.

PfAs from two Member States were received, which proposed to include a notification to the registrants to consider examining gonadal cells, which in case of a positive result would give an indication that BADGE and/or its metabolites is able to reach the gonads and cause genotoxic effects. The evaluating MSCA agreed to this proposal. You did not comment on this proposal.

In two PfAs from Member States, the concern that DGEBA/BADGE may cause DNA cross-linking, which may affect the performance of the standard Comet assay was raised. One PfA proposed that in order to clarify this concern an additional set of slides for the Comet assay which are submitted to modified experimental conditions to be able to detect cross-linking should be requested. A specific positive control for detecting cross-linking was also proposed to be included. The evaluating MSCA modified the revised draft decision to include this request.

You commented on the Member State PfAs to the revised draft decision. In your comments on the additional modification of the Comet assay for cross linking you highlighted the lack of a

detailed protocol, lack of historical test data and the lack of a significant validation study for positive and negative controls from several different laboratories using the modified experimental conditions to detect cross-linking.

The evaluating MSCA took your comments into consideration and included under test specifications examples of modifications to the experimental test conditions (treatment by MMS or ionising radiations) that can be employed to detect cross linking in the Comet assay and references to reliable test protocols (given in the OECD 489 test guideline (2016). The evaluating MSCA also included examples of known cross-linkers, which can be used as positive controls.

The evaluating MSCA also notes that a reliable protocol for detecting cross-linking is described in the references to the Comet assay guideline (OECD 489, version 2016) and that some testing laboratories perform Comet assays with experimental modifications to detect cross-linking. Furthermore, the evaluating MSCA notes that the inclusion of a known cross-linking substance (such as hexamethyl phosporamide or cisplatin) as a concurrent positive control will enable the evaluating MSCA to conduct a reliable evaluation of the result of the study without the need for historical test data.

Due to the additional difficulties of performing a non-standard Comet assay the evaluating MSCA agreed to extend the deadline for the requested Comet assay to 18 months instead of 15.

A PfA from one Member State proposed to request a TGR assay instead of a Comet assay or to give you the choice between these two options. Another Member State commented that they would prefer a request for a TGR instead of the Comet assay and that they would like a stronger scientific rationale for requesting the Comet assay. The evaluating MSCA commented that the reason for requesting the Comet assay was due to its higher sensitivity and a similar specificity as the TGR as described in Kirkland and Speit (2008), who assessed the sensitivity and specificity of UDS, TGR and Comet for rodent carcinogens. The evaluating MSCA also commented that if a reliable Comet assay yields a negative result in somatic tissues then the concern for mutagenicity will have been resolved and no further testing is needed. However, because the Comet assay has not yet been validated for detecting germ cell mutagenicity further mutagenicity testing may be needed in case of a positive result in the Comet assay. The evaluating MSCA agreed to give you the choice between the Comet assay (including the modification for cross-linking) and the TGR assay.

You commented that you strongly support the decision to give you the choice of conducting either the modified Comet assay or the TGR assay.

You also commented on the request for a TGR assay and stated that several aspects of carrying out a TGR assay necessitate a deadline of 24 months. Your justification favours a prolongation of the testing deadline to 24 months. For further details, see section Deadline to submit the requested Information below.

Conclusion on the information request 1.1: Mutagenicity

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study using the main constituent of the registered substance subject to this decision, i.e. 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bisoxirane, BADGE, EC No 216-823-5; CAS No 1675-54-3:

1.1 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 and liver. 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) shall 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 shall then also be analysed.

OR

1.1 B *In vivo* mammalian alkaline comet assay; test method: OECD 489 in rats, oral route (gavage), in the following tissues: liver, glandular stomach and duodenum. The optimum sampling time(s) should be determined based on relevant kinetic data if available, but otherwise the default sampling times in OECD 489 should be employed. Two sets of slides shall be prepared and analysed, one set submitted to standard experimental conditions and one set submitted to modified experimental conditions that enable the detection of DNA-DNA and DNA-protein crosslinks (according to e.g. references 23, 36-39 in the TG 489 or Pant et al. 2015). A specific positive control for DNA cross linking effects shall be included.

In addition to a robust study summary of the requested study you choose to perform, you must provide a full study report of the requested study, including all relevant details of the study, in order to permit a clear conclusion regarding the results of the study to be drawn by the evaluating MSCA. The reason for requesting the full study report is that all study details relevant for the evaluation by the evaluating MSCA of the results of especially higher tier tests are not always available in robust study summaries only.

ENDPOINT 1.2 CSR Related requests

1.2.1 Revision of Section 5.11 in the CSR on calculation of overall assessment factors(AF) in the derivation of DNELs

Concern identified

There is a concern that the consequence of incorrect derivations of assessment factors (AFs) / Derived no effect levels (DNELs) may be that the human health is set at risk with e.g. incorrect advice with regard to risk management measures (RMMs) and operation conditions (OCs). DNEL setting is crucial in order to avoid underestimating the risk, having in consideration the possible serious effects of the substance; in the present case, the substance is suspected to be a mutagen.

Review of existing information

ECHA observes that you have based the overall assessment factors (AF) in the derivation of

the DNELs on ECETOC-derived default assessment factors used to calculate the DNEL from chronic oral and dermal NOAEL, and have thus not followed the recommendations of the REACH *"Guidance on information requirements and chemical safety assessment Volume 8, Chapter R.8: Characterisation of dose [concentration]-response for human health (version 2.1, November 2012)"* and have not provided a full justification for the derivation of DNELs in line with Annex I, 1.4.1.

The REACH *"Guidance on information requirements and chemical safety assessment"*, Volume 8, Chapter R.8, provides further details and specifically provides default factors which should be applied to derive DNELs in the absence of substance specific information.

Annex I, 1.4.1 of the REACH Regulation requires that the following factors shall, among others, be taken into account when deriving DNELs:
a) the uncertainty arising, among other factors, from the variability in the experimental information and from intra- and inter-species variation;
b) the nature and severity of the effect;
c) the sensitivity of the human (sub-)population to which the quantitative and/or qualitative information on exposure applies;
d) and that the DNELs reflect the likely route(s), duration and frequency of exposure.

Conclusion

You are given two options: You shall revise the DNELs for workers and the general population by applying the assessment factors recommended in the REACH Guidance that are appropriate in this case. Subsequently, you shall re-assess related risks.
In the alternative, you shall, in accordance with Annex I, 1.4.1, provide a full justification for the DNELs derived for systemic effects via the dermal route for workers (long-term exposure) and via the dermal and oral route provided in the chemical safety report by specifying how the following has been taken into account:

a) the uncertainty arising, among other factors, from the variability in the experimental information and from intra- and inter-species variation;
b) the nature and severity of the effect;
c) the sensitivity of the human (sub-)population to which the quantitative and/or qualitative information on exposure applies;
d) and that the DNELs reflect the likely route(s), duration and frequency of exposure.

1.2.2. Further specifications to workers on the use of personal protective equipment

Concern identified

The registered substance may pose a serious risk for human health, having in consideration that the substance is suspected to be a mutagen and is classified as a skin sensitizer and dermal exposure should be reduced. Hence, further information on specifications of the advised PPEs is needed from you in order for the evaluating MSCA to be able to draw adequate conclusions relating to the potential unacceptable risk to human health.

A concern is raised if workers are not properly informed to use the right type of personal protective equipment (PPE; gloves, goggles, masks and coveralls) to protect themselves against exposure to chemicals. The use of unsuited material may even result in higher level of

exposure, than not using any protection at all, as the inside of contaminated gloves, may be covered with migrated substance – and the skin inside a glove is often humid – corresponding to exposure under occlusion.

Review of existing information

Your advice is for the workers to "wear chemically resistant gloves (tested to EN374) in combination with basic training" - without any further details. Furthermore, several processes are described where the advice of use of additional protection seems to be appropriate in e.g. manual spray applications.

ECHA notes that pursuant to Annex VI, section 5 of the REACH Regulation the information provided in the registration dossier must be consistent with that in the Safety Data Sheet. The requirements of Safety Data Sheets are specified in Annex II to the REACH Regulation (amended by Commission Regulation (EC) No 453/2010). According to section 8.2.2.2. of Annex II, "*detailed specifications shall be given on which equipment will provide adequate and suitable protection*".

Personal protective equipment (PPE: gloves, goggles and protection) specification is a requirement of Annex II, section 8.2.2. and the efficacy is needed to assess residual exposure occurring to workers when PPE are used. In Annex I, section 5.2.4. it is stipulated that "the estimation of the exposure level ... shall take into account (...) implemented and recommended risk management measures, including the degree of containment."

Conclusion

You are requested to provide further specification on personal protective equipment and the duration of use for all scenarios where the use of personal protective equipment is advised accordingly.

In particular the type of material, thickness and breakthrough times of the gloves and the duration of use for all exposure scenarios where the use of gloves is advised must be specified. Furthermore, you are requested to add information on sufficient protection of the body (coverall) or to justify why this is not advised in the relevant exposure scenarios, e.g. for manual spraying.

Consideration of registrants' comments on the initial draft decision regarding request 1.2: CSR

Concerning request 1.2.1: You have indicated your intention to provide details and justification to support the AF's in a future update of the dossier. However, you maintain that you consider ECETOC report #93 as a valid basis for the calculation of AFs au lieu of the ECHA guidance. ECHA maintains the request.

Concerning request 1.2.2: You committed to include more detailed specification on the use and type of PPE for workers in a future update of the dossier. ECHA appreciates the planned update. ECHA maintains the request until the update is performed and assessed.

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Deadline to submit the requested Information

In the initial draft decision, the time indicated to provide the requested information was 15 months from the date of adoption of the decision. In your comments on that initial draft decision you requested an extension of the timeline to 18-24 months. You sought to justify this request by shortage in laboratory capacity to perform the initially requested study under the former point 1.1: Standard Comet assay. Also, you pointed out that the comet assay was a technically challenging test. The argument of capacity shortage is not accompanied by any documentation from testing facilities. The arguments provided were not accepted and the deadline was initially not extended for performance of the standard Comet assay.
The decision was subsequently modified with respect to the Comet assay – which is now option 1.1 B. The request includes for two sets of samples to be taken, one standard and one to be treated to detect possible cross-linking. Due to the requested addition of the modification to the Comet assay, the deadline for reporting has been extended with 3 months, to 18 months.

In case option 1.1 A - the standard TGR assay (OECD 488) - is chosen, the time indicated to provide the requested information was 21 months from the date of the decision. In your comments to PfAs, you have provided arguments, supported by a document from a Contract Laboratory Organisation that performance and reporting of this test would take up to 24 months. Therefore, ECHA extended the deadline of the decision to 24 months, should option 1.1 A including the standard TGR assay OECD 488 be preferred by the registrants.

References

ECHA (2012).Guidance on information requirements and chemical safety assessment: Chapter R.8: Characterisation of dose [concentration]-response for human health http://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf

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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 human health from suspected mutagenicity; potential endocrine disruptor; exposure due to wide dispersive use, consumer use, high (aggregated) tonnage, Reaction product: bisphenol-A-(epichlorhydrin); epoxy resin (number average molecular weight ≤ 700) (DGEBA), CAS No 25068-38-6, EC No 500-033-5, was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015. The Competent Authority of Denmark (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In accordance with Article 45(4) of the REACH Regulation the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

The evaluating MSCA considered that further information was required to clarify the following concerns:
1. mutagenicity and
2. exposure to humans.

Therefore, it prepared a draft decision under Article 46(1) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 17 March 2016.

The decision making followed the procedure of Articles 50 and 52 of the REACH Regulation as described below.

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

Registrant(s)' commenting phase

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

The evaluating MSCA took the comments from you, which were sent within the commenting period, into account and they are reflected in the reasons (Appendix 1).
Your comments to Appendix 3, point 6 "*Notes for consideration to the Registrant(s) regarding the concern for endocrine disrupting properties of the registered substance"* are addressed in that note.

One information requirement in the initial draft decision was deleted on basis of your comments, whilst the remaining requests and the deadline were initially not 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).

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

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

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 in its MSC-53 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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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 fulfil otherwise 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 substance to be used should be the main constituent of the registered substance subject to this decision, i.e. 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bisoxirane, BADGE, EC No 216-823-5; CAS No 1675-54-3. It is the responsibility of all the Registrant(s) to agree on the source and specification of the test material to be used and to document the necessary information on composition of the test material. The substance identity information on the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing requested in this decision.

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 is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at: https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

   Further advice can be found at: http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.

5. Notes for consideration to the Registrant(s) regarding the request to perform testing under point 1.1 B

   Considerations on current limitations of the Comet Assay to be taken into account by the Registrant

   As reflected throughout the current version of the OECD 489 (April 2014) test guideline, several critical parameters pertaining to the transient nature of strand breaks, application of negative and positive controls, and technical parameters of gel electrophoresis affect the results of the Comet Assay and must be carefully controlled and documented (please see also 'Acceptability Criteria' (paragraph 58-65) and Annex 3: Current Limitations of the Assay' in the OECD 489).

Due to the transient nature of strand breaks the optimum sampling time is critical. (OECD 489, Annex 3 Current limitations of the Assay, paragraph 1). Also, the length of time from euthanasia to removal of tissues may be critical for the detection of strand breaks (OECD 489 paragraph 19). In order to maximize the ability to detect short-lived lesions animals should be euthanized and tissues collected at or soon after Tmax is reached. The laboratory needs to be proficient in harvesting multiple tissues from a single animal. Historical and contemporary positive and vehicle control data should be included in the study report. The scoring of cells must be done quantitatively using an automated or semi-automated image-analysis system.

The Comet Assay Working Group does not currently recommend the use of frozen samples in the *in vivo* comet assay, which means that samples from multiple tissues must be processed right away. (OECD 489, Annex 3 Current limitations of the Assay, paragraph 5).

Testing of gonadal cells in the Comet assay

You may consider examining gonadal cells in the Comet assay, as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation. Currently, the Comet Assay (OECD 489) is not validated to measure DNA strand breaks in mature germ cells (paragraph 10, OECD 489).

Consequently, the Comet assay is not equivalent to the TGR assay for the identification of germ cell mutagens and in particular it is not appropriate to use negative test results to conclude that a substance does not induce mutations in germ cells.

6. Notes regarding the concern for endocrine disrupting properties of the registered substance

Originally, the draft decision contained further notes for consideration to the Registrant(s) on the identified concern for endocrine disrupting properties of the registered substance. The registrant submitted several comments to the draft decision on this endpoint and also requested that the reference to the possibility of a future follow up request for an EOGRTS should be removed. The evaluating MSCA consequently removed the section relating to a concern for endocrine disruption since this is not relevant for the information requested in the present decision. It is important to note, however, that the evaluating MSCA does not consider this concern as clarified and that this concern therefore may potentially be addressed in a second draft decision at a later stage.