Ministry of Environment of Denmark
Environmental Protection Agency

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Substance name

2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bisoxirane

EC No 216-823-5 (previously 500-033-5)

CAS RN 1675-54-3 (previously 25068-38-6)

Evaluating Member State: **Denmark**

Dated: 20 May 2021

Evaluating Member State Competent Authority

MSCA name: Danish Environmental Protection Agency
Tolderlundsvej 5
5000 Odense C
Denmark
Tel: +45 72544000
Email: mst@mst.dk
Contact person: Lea Stine Tobiassen
Tel: +45 22349873
E-mail: lesto@mst.dk

Year of evaluation in CoRAP: 2015

Before concluding the substance evaluation a Decision to request further information was issued on: 19 May 2017

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process, the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] CoRAP: http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)] bisoxirane, EC no 216-823-5 (CAS RN 1675-54-3) (referred to as BADGE below), previously registered as "Reaction product: bisphenol-A-(epichlorhydrin); epoxy resin (average molecular weight ≤ 700), EC no 500-033-5 (CAS RN 25068-38-6), was originally selected for substance evaluation in order to clarify concerns about:

1) Suspected CMR
2) Potential endocrine disruptor;
3) Exposure/Wide dispersive use, consumer use, high (aggregated) tonnage

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A compliance check on substance idenfication was performed upon request of the eMSCA in 2016, leading to the change in the substance identity.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	√

Based on the available data, the eMSCA has concluded that no further information should be requested. A residual concern for one of the endpoints of concern, endocrine disruption subsists. However, no tests to clarify the concern are feasible and proportionate at this point.

Also, no further regulatory risk management measures are suggested.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

Not relevant.

4.1.1. Harmonised Classification and Labelling

Not relevant.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not relevant.

4.1.3. Restriction

Not relevant.

4.1.4. Other EU-wide regulatory risk management measures

Not relevant.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/exposure	√
Actions by the registrants to ensure safety, as reflected in the registration dossiers(e.g. change in supported uses, applied risk management measures, etc.)	

The evaluation clarified the original concern for mutagenicity, which was based on several positive results for gene mutation and chromosomal aberration tests *in vitro* including the Ames test, mammalian chromosomal aberration test and the micronucleus test.

In vivo however, BADGE was negative in chromosomal aberration tests and at the initial site of contact in the transgenic rodent somatic and germ cell gene mutation assay requested in this substance evaluation. Based on this, the eMSCA concludes that BADGE is not regarded to be mutagenic.

Based on the available information, the concern for the possible carcinogenicity of BADGE is clarified, and BADGE is considered not to be carcinogenic.

With respect to the concern for endocrine disrupting effects in humans the available information from QSARs indicated some endocrine activity, but in *vitro* and *in vivo* data were insufficient to confirm the concern. However, the eMSCA notes that interpretation of the *in vitro* data was difficult due to confounding high cytotoxicity in the media used. The results from the available older OECD compliant mammal studies show only slight effects on the endocrine organs at relatively high doses and the concern for endocrine disruption in mammals is therefore reduced. However, as the studies were all conducted before the inclusion of ED relevant end-points in according to recent versions of OECD test guidelines, the studies miss a number of ED sensitive endpoints, leaving a residual concern for endocrine activity of BADGE. Nevertheless, having regard to animal welfare, the eMSCA evaluates that a request for further *in vivo* studies in mammals would be disproportionate.

The original concern for endocrine disrupting effects in the environment based on *in vitro* and *ex vivo* results have not been clarified. Due to the high toxicity of the compound in fish, it is assessed that measurements of endocrine activity can most likely not be confirmed in currently available recognised test guidelines.

With regards to the concerns related to exposure, an unusually high level of protection from protective gloves (99%) is applied in the calculation of RCR values in the dossier for professional use. In addition, it is assumed that all consumers will use gloves. The eMCSA expects that information on how to achieve this high level of protection and how to ensure glove use by all consumers while handling the substance is thoroughly passed on through the supply chain.

In conclusion, the eMSCA considers that the concerns raised in the substance evaluation are clarified to the extent currently possible, and that no further testing or regulatory action is needed.

5.2. Other actions

Not relevant.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Not relevant.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bisoxirane, EC number 216-823-5 (CAS RN 1675-54-3) was selected for substance evaluation in order to clarify concerns about:

1) Suspected CMR;
2) Potential endocrine disruptor;
3) Exposure/Wide dispersive use, consumer use, high (aggregated) tonnage

Table 4

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Suspected CMR (Mutagenicity and carcinogenicity)	Concern for mutagenicity clarified in follow-up evaluation: not mutagenic. No further testing proposed. Concern for carcinogenicity clarified: not carcinogenic
Endocrine Disruption	Residual concern for endocrine disrupting effect. No further request or regulatory action proposed.
Human exposure	a) Concern for DNEL setting appropriately addressed in follow-up. b) Concern for exposure adressed by requirement from registrant to use of personal protective equipment (gloves) with high protection factor for workers

A summary of the evaluation is given above in section 5.1. For details of evaluation, see individual sections below 7.9.5, 7.9.6, 7.9.9, 7.10 and 7.12.1.

7.2. Procedure

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 and carcinogenity; potential endocrine disruptor; exposure due to wide dispersive use, consumer use and high (aggregated) tonnage, the registered substance: reaction product: bisphenol-A-(epichlorhydrin); epoxy resin (number average molecular weight ≤ 700), DGEBA (EC No 500-033-5, CAS RN 25068-38-6), was included on 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.

Pursuant to 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 eMSCA evaluated the information given by the applicant in the joint registration on the registered substance – at the time DGEBA (EC no 500-033-5) provided as an aggregated IUCLID registration dossier from ECHA in April 2015, and updated by the registrant in August 2015. The dossier information was supplemented with information obtained from the registrant and scientific information from publicly available sources. Also, results from QSAR modelling were included by the eMSCA.

The eMSCA reviewed the following endpoints: Substance identity, Mutagenicity, Carcinogenicity, Endocrine disruption, including relevant information from repeated dose and reproductive toxicity studies as well as studies of fate and effects in the aquatic environment, exposure scenarios and DNEL setting.

No other endpoint was investigated in this substance evaluation.

A meeting with the registrants representatives was held in May 2015, and continuous communication by e-mail on the dossier elements was ongoing throughout the evaluation.

The eMSCA prepared the present draft SEV and a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 17 March 2016.

Following comments from the applicant, a revised draft decision was elaborated by the eMSCA. The MSC commenting process resulted in the adoption (through written procedure) of the decision in May 2017.

The substance evaluation decision included the following requests:

1. Mutagenicity: The choice was given to the registrant(s) to perform either:

a) 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, analysing the glandular stomach, duodenum and liver or
b) an *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 mutagenicity study preferred by the registrants was to be conducted on BADGE, EC No 216-823-5; CAS RN 1675-54-3, which at the time was the main constituent of the registered substance.

2. CSR-related requests:

a) Revision of Section 5.11 in the CSR on calculation of overall assessment factors (AF) in the derivation of DNELs or including a substance specific justification for the AFs used, and
a) Further specifications to workers on the use of personal protective equipment.

The decision included deadlines for the updated dossier of 27 May 2019 in case the TGR was performed, or 26 November 2018 in case the registrant(s) chose to perform a Comet assay.
A note on the concern for endocrine disruption was included in the decision.

The substance evaluation decision also included a paragraph on possible deviation for the registrered substance from the definition of a mono-constituent substance.
A targeted compliance check was therefore performed, and a communication on required changes to substance identify was issued on 14 December 2016.
A revised registration was uploaded 21 March 2017 correcting the substance identity information, including a change of the registered substance name and identifiers to 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxy-methylene)]bisoxirane, BADGE (EC No 216-823-5, CAS RN 1675-54-3).

In response to the substance evaluation the registrant chose to perform the test under option 1a) and an updated registration was updated 2 May 2019. Due to descrepencies from the request in the conduct of the study, the eMSCA requested further information from the registrant. A revised registration was filed on 24 September 2019 including further details on the TGR study, and the follow-up evaluation was launched.

The eMSCA evaluated that despite residual concerns on endocrine disruption, the substance evaluation would be concluded with no further information requirements, and that a conclusion document would conclude the evaluation.

The present final conclusion document was filed in May 2021.

7.3. Identity of the substance

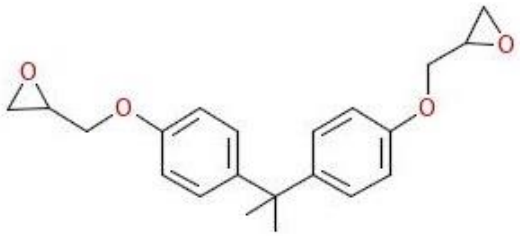
Table 5

SUBSTANCE IDENTITY	
Public name:	2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bisoxirane Previously on CoRAP as reaction product: bisphenol-A-(epichlorhydrin); epoxy resin (number average molecular weight ≤ 700), DGEBA
EC number:	216-823-5 (previously on CoRAP as EC: 500-033-5)
CAS registry number:	1675-54-3 (previously registration CAS RN 25068-38-6)
Index number in Annex VI of the CLP Regulation:	603-073-00-2
Molecular formula:	C21H24O4
Smiles notation	CC(C)(C1=CC=C(OCC2CO2)C=C1)C1=CC=C(OCC2CO2)C=C1
Molecular weight range:	340.0 ≤ MWR ≤ 700.0
Synonyms:	BADGE

Type of substance:

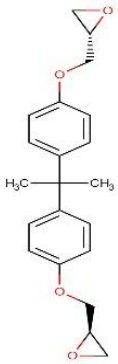
☐ Mono-constituent ☒ Multi-constituent ☐ UVCB

Structural formula:



Composition:

Main constituent 1:

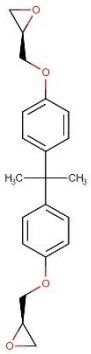


Reference substance name: meso-2-{[4-(2-{4-[(oxiran-2-yl)methoxy]phenyl} propan-2-yl)phenoxy]methyl}oxirane

Molecular formula: C21H24O4

IUPAC Name: meso-2-{[4-(2-{4-[(oxiran-2-yl)methoxy]phenyl}propan-2-yl) phenoxy]methyl}oxirane

Main constituent 2:



Reference substance name: (2RS)-2-({4-[2-(4-{[(2RS)-oxiran-2-yl]methoxy}phenyl)propan-2-yl]phenoxy} methyl)oxirane

Molecular formula: C21H24O4

IUPAC Name: (2RS)-2-({4-[2-(4-{[(2RS)-oxiran-2-yl]methoxy}phenyl)propan-2-yl]phenoxy}methyl)oxirane

Impurities:

Identity and concentrations of impurities are confidential information only available in the confidential IUCLID dossier.

7.4. Physico-chemical properties

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	liquid at 20°C and 101.3 kPa
Vapour pressure	0.000000046 Pa at 25 °C
Water solubility	6.9 mg/L at 20 °C
Partition coefficient n-octanol/water (Log Kow)	Log Kow (Pow): 3.242 at 25 °C
Flammability	**Endpoint waived:** The epoxy resin substance does not ignite when it comes into contact with water.

Explosive properties	*NA*
Oxidising properties	**Endpoint waived:** In accordance with Column 2 of REACH Annex VII, the Oxidising properties study (required in Section 7.13) does not need to be conducted as the substance is incapable of reacting exothermically with combustible materials, for example on the basis of the chemical structure (e. g. organic substances not containing oxygen or halogen atoms and these elements are not chemically bonded to nitrogen or oxygen, or inorganic substances not containing oxygen or halogen atoms).
Granulometry	**Endpoint waived:** In accordance with column 2 REACH Annex VII, the granulometry study (required in section 7.14) does not need to be conducted as the substance is marketed or used in a non solid or granular form.
Stability in organic solvents and identity of relevant degradation products	The substance is soluble and stable in the organic solvents dimethyl sulphoxide. The substance is also stable in solvents such as xylene, acetone and methyl ethyl ketone.
Dissociation constant	**Endpoint waived:** The substance cannot dissociate due to a lack of relevant functional groups. The dissociation constant for the substance is irrelevant. The UV spectra do not show a shift with change of pH confirming that the substance does not contain a relevant functional group.

7.5. Manufacture and uses

7.5.1. Quantities

Table 8

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000- 50,000 t
☐ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☒ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 9

USES

Uses as intermediate	
Formulation	F-2: Formulation Industrial **Environmental release category (ERC):** ERC 2: Formulation of preparations **Process category (PROC):** PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)
Uses at industrial sites	IW-3: Industrial use as monomer **Environmental release category (ERC):** ERC 4: Industrial use of processing aids in processes and products, not becoming part of articles **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities **Technical function of the substance during formulation:** Intermediates monomer for the production of polymers IW-4: Industrial use as intermediate **Environmental release category (ERC):** ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates) **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities **Product Category used:** PC 19: Intermediate **Technical function of the substance during formulation:**

	Intermediates IW-6: Use on industrial site **Environmental release category (ERC):** ERC 5: Industrial use resulting in inclusion into or onto a matrix **Process category (PROC):** PROC 7: Industrial spraying PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring PROC 15: Use as laboratory reagent **Technical function of the substance during formulation:** Binding agents
Uses by professional workers	PW-5: Professional use **Environmental release category (ERC):** ERC 8c: Wide dispersive indoor use resulting in inclusion into or onto a matrix ERC 8f: Wide dispersive outdoor use resulting in inclusion into or onto a matrix **Process category (PROC):** PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 10: Roller application or brushing PROC 11: Non industrial spraying PROC 13: Treatment of articles by dipping and pouring PROC 19: Hand-mixing with intimate contact and only PPE available. PROC 24: High (mechanical) energy work-up of substances bound in materials and/or articles **Technical function of the substance during formulation:** Binding agents
Consumer Uses	**Environmental release category (ERC):** ERC 8c: Wide dispersive indoor use resulting in inclusion into or onto a matrix ERC 8f: Wide dispersive outdoor use resulting in inclusion into or onto a matrix **Product Category used:** PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 9b: Fillers, putties, plasters, modelling clay **Technical function of the substance during formulation:** Binding agents
Article service life	

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 10

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS RN**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Codes	**Hazard statement codes**		
603-073-00-2	bis-[4-(2,3-epoxipropoxi)phenyl]propane	216-823-5	1675-54-3	Skin Sens. 1 Skin Irrit. 2 Eye Irrit. 2	H317 H315 H319	Skin Irrit. 2; H315: C ≥ 5% Eye Irrit. 2; H319: C ≥ 5%	

7.6.2. Self-classification

In the registration(s):

The registrant(s) attributes the following classification:
Skin Irrit 3: H315: Causes skin irritation
Skin Sens, 1; H317: May cause an allergic skin reaction.
Eye irrit 2: H319: Causes serious eye irritation.
Aquatic chronic 2: H411: Toxic to aquatic life with long lasting effects.

Self-classifications:

The following hazard classes are notified among the 9 aggregated notification covering 916 notifiers in the C&L Inventory:

- Water reactive H261
- Aquatic Acute 1 H400
- Aquatic Chronic 3 H412

7.7. Environmental fate properties

Stability

Table 11 Hydrolysis

Method	Results	Remarks	Reference
EEC Test method A6 Similar to OECD Guideline 111 (Hydrolysis as a function of pH) Solubility in water was measured using a supersaturated solution which	<u>Solubility in water at 20°C:</u> 3.6 mg/L <u>Half-lives obtained:</u> T½ pH 4: 7h (50°C), 25.6h (38°C) T½ pH 7: 9h (50°C), 25.5 h (38°C)	2 (reliable with restrictions) Test material EPIKOTE 828	Unpublished report, 1993

Method	Results	Remarks	Reference
was left for 24 hours and centrifuged before directly analyzed for concentration of BADGE. 1.93 mg/l EPIKOTE 828 in buffered solutions at pH 4, 7, and 9. The test were performed at 38°C and 50°C. Analytical method: reverse-phase HPLC with UV detection.	T½ pH 9:8.5h (50°C), 32.5h (38°C) <u>Half-life (DT50) extrapolated based on Arrhenius equation to 25°C:</u> T½ (pH 4): 116h; rate constant: 0.006h^{-1}. T½ (pH 7): 86h; rate constant: 0.008h^{-1}. T½ (pH 9): 171h; rate constant: 0.004h^{-1}. T½ (overall): 117hours Transformation products: not measured		
Isolation and characterization of hydrolysis products Hydrolysis: A 400 mg/L suspension of EPIKOTE 828 in 20:80 (tetrahydrofuran: water) was heated in an oven at 70°C for 16-50 hours. Identification: HPLC with UV detection.	Two hydrolysis products were identified: Monodiol BADGE (3-[4-[1-[4-(2,3-epoxypropoxy)-phenyl]-1-methylethyl]phenoxy]propane-1,2-diol) (CAS RN76002-91-0) Bis(diol) BADGE (1,2-Propanediol, 3,3'-[(1-methylethylidene) bis(4,1-phenyleneoxy)]bis-3,3'-[(1-methylethylidene)bis-(4,1-phenyleneoxy)]bispropane-1,2-diol) (CAS RN 5581-32-8)	2 (reliable with restrictions) Test material: EPIKOTE 828 purified to >99% BADGE (1675-54-3).	Pérez-Lamela et al. (2016)

The registrant has included one study in the registration dossier on the hydrolysis of DGEBA; (unpublished, 1993) investigated this using EPIKOTE 828. Based on the authors suggestion the registrants stated that hydrolysis was independent of pH why the six measured hydrolysis half-lives were converted to one half-life of 117 hours at 25°C (95 CI: 68-204 h).

The eMSCA notes that the preliminary solubility test performed was done using a supersaturated solution, which was left for 24 hours and centrifuged before directly analysed for concentration of BADGE. As the hydrolysis of EPIKOTE 828 was found to be 86 h at neutral pH and 25°C this probably means that part of the substance was hydrolyzed as the half-life of the substance is only 3,5 times longer. In a study by Pérez-Lamela et al. (2016) it was argued that BADGE is poorly soluble in water based on the predicted logKow however as BADGE was hydrolyzed it became less hydrophobic.

The hydrolysis products were not identified in the study by Fisk (1993). These has been identified in a study by Pérez-Lamela et al. (2016) using EPIKOTE 828 purified to >99%. Two hydrolysis products were identified; monodiol BADGE (CAS RN 76002-91-0) and bis(diol) BADGE (CAS RN 5581-32-8).

The half-life based on hydrolysis for the registered substance was based on the study by Fisk (1993) set to be 117 hours at 25°C (95 CI: 68-204 h).

As the identification of hydrolysis products was performed using purified EPIKOTE 828 containing > 99% BADGE (CAS RN 1675-54-3) the hydrolysis products of the other constituents of the registered substance is unknown. Other constituents such as the first

oligomer (n=1, 2, 3) of DEGBA are expected to hydrolyze on a similar manor yielding the mono- and bis-diol hydrolyze products.

7.7.1. Degradation

Table 12: Screening test

Method	Results	Remarks	Reference
OECD Guideline 301 F (Ready biodegradability: Manometric respiratory test) Based on previous experience with an alkyl epoxy, inhibition of respiration was observed at 100 mg/l why the test material concentration was lowered to 20 mg/l. The test material was exposed to sewage treatment micro-organisms with culture medium in sealed culture vessels in diffuse light at 21 ± 1ºC for 28 days. The test material was adsorbed onto granular silica gel prior to dispersion in the test. The degradation of the test material was assessed by two methods; - the measurement of daily oxygen consumption values at Day 28. - compound specific analysis on Days 0, 7, 14, 21 and 28. Control solutions with inoculum and the standard material, aniline, together with a toxicity control were used for validation purposes. Identification: Gas chromatography	Under test conditions no biodegradation observed <u>Degradation of test substance based on oxygen consumption:</u> 5% after 28 d (O2 consumption) <u>Degradation in aniline toxicity control based on oxygen cons.:</u> 64% after 14 d 71% after 28 <u>Degradation in abiotic control (sodium azide) based on oxygen cons.:</u> 30% after 14 d 57% after 28 <u>Degradation of test substance using compound specific analysis:</u> 10-13% at 0 d 10% after 7 d 37% after 14 d 46% after 21 d 85% after 28 d <u>% Degradation in abiotic control (sodium azide) using compound specific analysis:</u> 91% after 28 d	1 (reliable without restrictions) Test material: EPIKOTE 828*	Unpublished report (2010)

Method	Results	Remarks	Reference
OECD Guideline 301B (Ready biodegradability: CO2 Evolution test) Bacteria obtained from sewage treatment plant. The volume of the test solution was reduced from 3L to 1.5L. The sludge was aerated for 4 hours, settled, and decanted. The supernatant was aerated for a further 48 hours and used as inoculum for the test.	Not considered biodegradable. Degradation after 28 days: 10 mg/L = 6% 20 mg/L = 12%	1 (reliable without restrictions) TK 10490* commercial grade	Unpublished report (1985)
Test type: BOD, TOD and COD. BOD's were set using Michigan Division Wastewater Treatment Plant effluent as seed material. ThOD was calculated from the total oxidation of the pure compound. TOD (TOD-Dow analyzer) and COD ("powder" potassium dichromate) were also determined. Initial conc.: 10 mg/L Based on: ThOD/L	BOD: 0 g O2/g test mat No decrease in oxygen on day 5, 10, or 20. Conclusion: D.E.R. 331 consumed no oxygen in the standard BOD test.	supporting study 2 (reliable with restrictions) Test material: D.E.R. 331*	Unpublished report (1976)

*The code names used refer to the substance: 4,4'-isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane (CAS 25068-38-6).

The test material has been tested for biodegradation in an enhanced ready test following the OECD 301F (oxygen depletion) design. Test material specific analysis revealed a decrease in the concentration of the test substance with formation of the mono- and bis-diol transformation products. At termination of the study (day 28) 15% of the initial measured concentration could be recovered. The same decrease in test material was observed in abiotic control (sodium azide) indicating that this is not due to a biological process. This was supported by the results on oxygen consumption where only 5% of the test material was mineralized after 28 days.

A modified Sturm test (OECD 301B) at 10 and 20 mg DOC/L (approx 13 and 27 mg test material/L) also indicated a limited mineralization of 6 and 12%, respectively. This was attributed to the low solubility of the test material by the registrants (unpublished report, 1985). Likewise, BOD testing at 10 mg/L ThOD (approx 4 mg/L test material) showed no decrease in Oxygen at day 5, 10 and 20 (TDCC, 1975).

The first order rate of elimination in activated sludge at 20 °C is 0.0565 d^{-1}.

Summary on degradation

The material has shown limited mineralization in screening test for ready biodegradability at concentrations exceeding expected environmental concentrations. It is not expected that the biodegradation rate will exceed the rate of hydrolysis ($t_{½}$=5 d at 25°C).

Products of hydrolysis for BADGE (CAS RN 1675-54-3) are well characterized as mono- and bisdiol transformation products, however their fate is unknown. Other constituents such as the first oligomer (n=1, 2, 3) of DEGBA are expected to hydrolyze on a similar manor yielding the mono- and bis-diol hydrolysis products.

7.7.2. Environmental distribution

Not evaluated.

7.7.3. Bioaccumulation

Not evaluated.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

No evaluated.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Toxicokinetics (absorption, distribution, metabolism and excretion)

The results of studies on absorption, distribution, metabolism and excretion are summarized in the following table:

Table 13 Studies on absorption, distribution, metabolism and excretion

Method	Results	Remarks	Reference
Comparison of the fate of a single dermal application and of a single oral dose of [^{14}C]-DGEBPA in the mouse Mouse (CF-1), male, (n=6 pr. group) Single dermal application of approximately 56 mg/kg bw [^{14}C]-DGEBPA. Urine and feces were collected daily. The mice were killed in groups of two, at 1, 3 and 8 days after treatment. The intestines were removed intact and samples of liver, kidney, fat and blood were taken. The skin was removed from the remaining carcass. Single oral dose by gavage of approximately 55 mg/kg bw [^{14}C]-DGEBPA. Samples were	Dermal application: The percutaneous absorption of DGEBPA was slow. About 90% of the radioactivity was recovered from the skin and foil covering the application area 24 hours after application. This figure fell to about 40% after 8 days. The total radioactivity excreted in urine and feces after 8 days was approximately 5% and 41% of the administered dose, respectively. The recovery of radioactivity in the intestine, liver, kidney and blood after 8 days was below 1% of the administered dose. Oral dosing: Excretion of the radioactivity was very rapid being >88% of the administered dose within two days. The total radioactivity excreted in urine and feces after 8 days was approximately 10% and 80% of the administered dose, respectively. The recovery of radioactivity in the intestine, liver, kidney and blood after 8 days was below 1% of the administered dose.	Original study report not available to eMSCA. 2 (reliable with restrictions) Key study Study appears to be conducted on BADGE, although named DGEBPA purity of >99%, labelled at the isopropylidene methylene carbon.	Climie et al. (1981a)

Method	Results	Remarks	Reference
taken as described above. Metabolites in urine and feces were identified by thin layer chromatography (TLC)	Similar metabolic profiles in urine and feces were observed after oral administration and dermal application (0-24 hours post-dosing). The parent compound was the major component identified (97% following dermal application; no figure presented for oral administration in the article).		
Identification of metabolites in urine and feces following a single oral dose of [^{14}C]-DGEBPA in the mouse Mouse, male Experiment 1: Six mice (CF-1) were dosed orally with [^{14}C]-DGEBPA (approximately 55 mg/kg bw). Urine and feces were collected daily for eight days. Experiment 2: Six mice (CD) were dosed orally with [^{14}C]-DGEBPA (approximately 715 mg/kg bw). Urine and feces were collected daily for two days. Experiment 3: Three mice (CF-1) were dosed orally with [^{14}C]-DGEBPA (approximately 55 mg/kg bw). Urine and feces were collected daily for three days. Experiment 4: Three mice (CF-1) were dosed orally with [^{14}C]-DGEBPA (approximately 215 mg/kg bw). Urine and feces were collected daily for two days. Experiment 5: Two mice (CF-1) were dosed orally with [^{14}C]-DGEBPA (approximately 88 mg/kg bw). Urine and feces were collected daily for four days. Metabolites in urine and feces were identified by TLC	The major metabolic pathway was hydrolytic opening of the two epoxide groups to form the bis-diol of DGEBPA (named 'III'). This metabolite was excreted in urine and feces (in both free (6%) and conjugated (2%) forms, totaling 8% of the administered dose), whereas the majority was further metabolized to various carboxylic acids. One of the carboxylic acids (named 'VIII') resulted from direct biochemical oxidation of one of the diol groups in 'III' and was the major metabolite excreted in feces (in both free (24%) and conjugated (3%) forms, totaling 27% of the administered dose). Another of the carboxylic acids (named 'VI') resulted from oxidative cleavage of one of the diol groups in 'III' or by oxidative decarboxylation of 'VIII' and was excreted in feces (in both free (14%) and conjugated (1%) forms, totaling 15% of the administered dose). Two other (unexpected) carboxylic acids (named 'VII' and 'V') were identified. They contained a 3-methylsulphonyl-2-hydroxypropoxy side chain in place of the 2,3-dihydroxypropoxy side-chain on the opposite end of 'VIII' and 'VI', respectively. These metabolites were excreted in feces (4% and 5% of the administered dose, respectively). The phenol diol of DGEBPA (named 'IV') was identified in the urine and feces (excreted in both free (4%) and conjugated (1%) forms, totaling 5% of the administered dose). This metabolite could be the result of oxidative dealkylation of DGEBPA or of the bis-diol ('III'). The three-carbon unit side-chain of DGEBPA is released either as glyceraldehyde (named 'XII') or as glycidaldehyde (named 'XIII'). Bisphenol-A was not detected in the urine or feces in either the free or conjugated form.	2 (reliable with restrictions) Key study [^{14}C]-DGEBPA: radiochemical purity of >99%, labelled at the isopropylidene methylene carbon Study appears to be conducted on BADGE, although named DGEBPA	Climie et al. (1981b)
Fate of DGEBA in male rats Rat (Fischer 344), male, (n=1 pr. treatment) Single oral dose by gavage of 2.7 mg/kg bw [^{14}C]-DGEBA Single intraveneous (iv) dose	The total recovery of radioactivity was low in the non-bile cannulated animals following oral (66%) and iv (52%) administration; in the bile cannulated animal, the total recovery was 131%. The feces was the primary route of excretion following oral (53%) and iv	2 (reliable with restrictions) supporting study [^{14}C]-DGEBA: radiochemical purity of 98%,	Unpublished report (1981a)

Method	Results	Remarks	Reference
of 0.43 mg/kg bw [^{14}C]-DGEBA Bile cannulated rat, single iv dose of 0.43 mg/kg bw [^{14}C]-DGEBA Blood, urine, feces and bile (bile cannulated animal only) samples were collected at the following intervals after administration: Blood: 0.5, 1.0, 1.7, 3, 4, 6, 8, 10, 12, 18 and 24 hours Urine: 6, 12 and 24 hours Feces: 24 hours Bile: 1, 2, 3, 6, 12 and 24 hours The liver, lungs, kidneys and intestines were removed from non-bile cannulated animals. Plasma radioactivity was detected by HPLC Metabolites were identified by TLC	(43%) administration; in the bile cannulated animal, the excretion was 3% in the feces and 86% in the bile. Urine was a minor route of excretion (8-9%) in non-bile cannulated animals; in the bile cannulated animal, the excretion in urine was 42%. The content in organs and tissues was low (1-2%). The absorption of radioactivity following oral administration was rapid ($t_{1/2}$ = 0.7 hour). The highest plasma ^{14}C-concentration was observed 4 hours after dosing. The radioactivity was cleared from the plasma in an apparent first-order manner following both oral and iv administration; however, the radioactivity was cleared from the plasma faster following oral administration than following iv administration. Less than 10% of the radioactivity in the plasma was identified as [^{14}C]-DGEBA following oral administration. Different metabolites were excreted following oral and iv administration. The parent compound was not excreted in either the urine or bile. The major metabolite found in the bile was also found in the urine following iv administration, but not following oral administration. Metabolites found in the urine following oral administration were not found in either the bile or urine following iv administration. The stability of [^{14}C]-DGEBA in simulated gastric and intestinal fluid was investigated. The half-life for the degradation of [^{14}C]-DGEBA in simulated gastric fluid was estimated to 70 minutes. [^{14}C]-DGEBA was not rapidly hydrolyzed at pH's found in the small intestine.	labelled at the isopropylidene methylene carbon Study appears to be conducted on BADGE although named DGEBA	
Urinary excretion of DGEBPA in male rabbits Rabbit (New Zealand white), male, (n=3 pr. group) Single oral dose of 5 or 50 mg/kg bw DGEBPA, in gelatin capsule Free and sulfate conjugated forms of the bis-diol metabolite of DGEBPA in urine were identified by HPLC	The excretion of the free bis-diol metabolite in urine was low with about 2% of the dose following administration of 5 mg/kg bw and about 0.4% following 50 mg/kg bw. The majority of the bis-diol metabolite excreted in the urine was in the conjugated form with the amount of total (free and conjugated forms) of about 10% (range 2.7-16.7%) of the dose following administration of 5 mg/kg bw and about 2% following 50 mg/kg bw.	2 (reliable with restrictions) Key study	Unpublished study (1983)
Metabolic inactivation of BADGE in lung and liver of	GSH conjugation:	2 (reliable with	Boogaard et

Method	Results	Remarks	Reference
humans, rats and mice *in vitro* Rates of epoxide hydrolase (EH) catalyzed hydrolysis and glutathione S-transferase (GST) catalyzed conjugation with glutathione (GSH) were studied in subcellular fractions of liver and lung of human, Fisher 344 rat and C3H mouse The identity of the hydrolysis products formed was confirmed by co-elution on HPLC with the synthesized reference compounds and by comparison of the mass spectra obtained by LC-MS.	Both a small degree of chemical reaction and some inconsistent enzyme-catalyzed GSH conjugation of BADGE were observed. Hydrolysis: BADGE was rapidly hydrolyzed to the corresponding (di)glycerol ethers upon incubation with either cytosolic or microsomal fractions. The EH- catalyzed hydrolysis was linear with time for at least 10 minutes and linear with protein concentrations up to 1.5 mg/ml for liver and 3.0 mg/ml for lung subcellular fractions. All data were based on 10 minutes incubations and protein concentrations of 1.0 mg/ml for liver and 0.4-2.0 mg/ml for lung preparations. In general, the V_{max}/K_m ratio for microsomal EH was higher than for cytosolic EH. The V_{max}/K_m in the liver cytosol fraction was 117, 32.6 and 198 in human, rat and mouse, respectively. In liver microsomes, the V_{max}/K_m was 239, 188 and 203 in human, rat and mouse, respectively. In lung cytosol, the V_{max}/K_m was 18.7, 7.8 and 13.6 in human, rat and mouse, respectively. In lung microsomes, the V_{max}/K_m was 157, 38.1 and 53.4, respectively.	restrictions) supporting study	al. (2000b)
Urinary bisphenol A in male workers exposed to BADGE and mixed organic solvents Cross sectional study with 42 workers whose job it was to spray epoxy resin hardening agents including BADGE and mixed organic solvents, and 42 matched control workers without BADGE use in the same plants. Urinary bisphenol A was measured by HPLC. Concentration of urinary metabolites (o,m,p-cresol, o,m,p-methylhippuric acid, 2-ethoxyacetic acid, 2-butoxyacetic acid, and methyl isobutyl ketone) were measured by HPLC or GC.	A significant difference in bisphenol A (BPA) concentrations was observed between the epoxy resin sprayers (median 1.06; range: not detected to 11.2 µmol/mol creatinine) and the controls (median 0.52; range: not detected to 11.0 µmol/mol creatinine). BPA was not detected for three epoxy resin sprayers and one control. Urinary metabolites of organic solvents were detected more frequently in the epoxy resin workers compared with the controls: o-cresol (81%, 33%), methylhippuric acids (62%, 12%), and 2-butoxyacetic acid (62%, 0%). Urinary metabolite concentrations were all higher in the epoxy resin workers compared with the controls. Urinary 2-ethoxyacetic acid and methyl isobutyl ketone were not detected in either group.	3 (not reliable) supporting study Substance is given as 'an epoxy resin hardening agent including BADGE and mixed organic solvents'	Hanaoka et al. (2002)
Urinary excretion of BADGE and its derivatives in human urine Urine samples were collected from healthy volunteers in the US (n=31), from Chinese adult volunteers (n=26) and from 9-	BADGE and the three derivatives (collectively referred to as BADGEs in the article) were found in all the urine samples analyzed. Total urinary concentrations of BADGEs in the US ranged from 1.24 to 9.03 ng/ml, with a GM concentration of 3 ng/ml. Concentrations of BADGEs in urine from adults (GM: 1.36 ng/ml) and children (1.02 ng/ml) in China	2 (reliable with restrictions) supporting study	Wang et al. (2012)

Method	Results	Remarks	Reference
10 year old children in China (n=70). Urine samples were analyzed for free and total (free plus conjugated) concentrations of BADGE and three derivatives, bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O], bisphenol A (3-chloro-2- hydroxypropyl) (2,3-dihydroxypropyl) ether [BADGE·HCl·H_2O], and bisphenol A bis (2,3-dihydroxypropyl) ether [BADGE·$2H_2O$], using HPLC-MS/MS).	were 3-fold lower than the concentrations found in the US. Both free and conjugated forms of BADGEs were present in urine, and the proportion of free form was inversely related to the total concentration of BADGEs. Among the four BADGEs measured in urine, BADGE·$2H_2O$ (the bis-diol of BADGE) was the predominant compound, accounting for 45-60% of the total BADGEs concentration, followed by BADGE (17-24%). The distribution of the four BADGEs varied, depending on age, gender, and ethnicity of the adults and children. Bisphenol A concentrations were also measured in urine; the concentrations of BADGEs in US urine were 3- to 4-fold higher than the corresponding concentrations of bisphenol A.		
Urinary excretion of BADGE and its derivatives in human urine Urine samples were collected from Athens, Greece (n=100; number of samples of females/males: 50/50). The target compounds were analyzed after enzymatic deconjugation of urine samples, followed by liquid–liquid extraction (LLE). The total concentrations of BADGE and four derivatives of BADGE were determined by HPLC-MS/MS.	BADGE and the four derivatives were found in urine, and the rank order of detection rate was: BADGE·$2H_2O$ (90%, the bis-diol of BADGE), BADGE·HCl·H_2O (19%), BADGE·H_2O (9%, the mono-diol of BADGE), BADGE (4%) and BADGE·HCl (3%).	2 (reliable with restrictions) supporting study	Asimakopoulos et al. (2014)

7.9.1.1.1. Absorption, distribution and excretion:

The following studies on absorption, distribution and excretion of BADGE in mice and rats have been included by the registrant in the toxicokinetic section of the CSR.

Following oral administration of BADGE to mice, BADGE was rapidly and extensively excreted (>88% of the administered dose within two days) with the fecal route being the major route of elimination (approximately 80% of the administered dose) while a small fraction of the administered dose was recovered in the urine (approximately 10% of the administered dose). The percutaneous absorption of BADGE was slow. The recovery of radioactivity in organs and tissues was low (<1% of the administered dose), except for the skin following dermal application. (Climie et al. (1981a), unpublished report (1978a)).

Similarly, BADGE was eliminated mainly by excretion via the feces following oral administration of BADGE to non-bile cannulated rats (53% of the administered dose) with urine as a minor route (8-9% of the administered dose). In the bile cannulated rat, the excretion was 3% in the feces, 86% in the bile and 42% in the urine. The recovery of radioactivity in organs and tissues was thus also low in rats (1-2% of the administered dose) (unpublished report, 1981a). It should be noted, that in this study, the total recovery of radioactivity was low in the non-bile cannulated animals (oral: 66%; iv: 52%) while in the bile cannulated animals, the total recovery was 131%.

No information on absorption, distribution and excretion of BADGE in other animal species, or in humans, has been included by the registrant in the toxicokinetic section of the CSR.

7.9.1.1.2. Metabolism:

Studies on metabolism of BADGE in mice, rats and rabbits, as well as in tissue fractions *in vitro*, as well as limited human information have been included by the registrant in the toxicokinetic section of the CSR. Further information from other sources are also described below.

In mice, similar metabolic profiles in urine and feces were observed after oral administration and dermal application of BADGE (97% pure following dermal application; no figure presented for oral administration in the article by Climie and coworkers, based on unpublished report, 1978a) – the original study report was not available to the eMSCA). (Climie et al., 1981a, unpublished report, 1978a).

The comprehensive metabolism studies in mice given a single oral dose of BADGE (Climie et al. (1981b), unpublished report (1979a)) showed that the major metabolic pathway in mice was hydrolytic opening of the two epoxide groups in BADGE to form the bis-diol of BADGE (III in Figure 1), which was predominantly further metabolized to various carboxylic acids, see Figue 1 below.

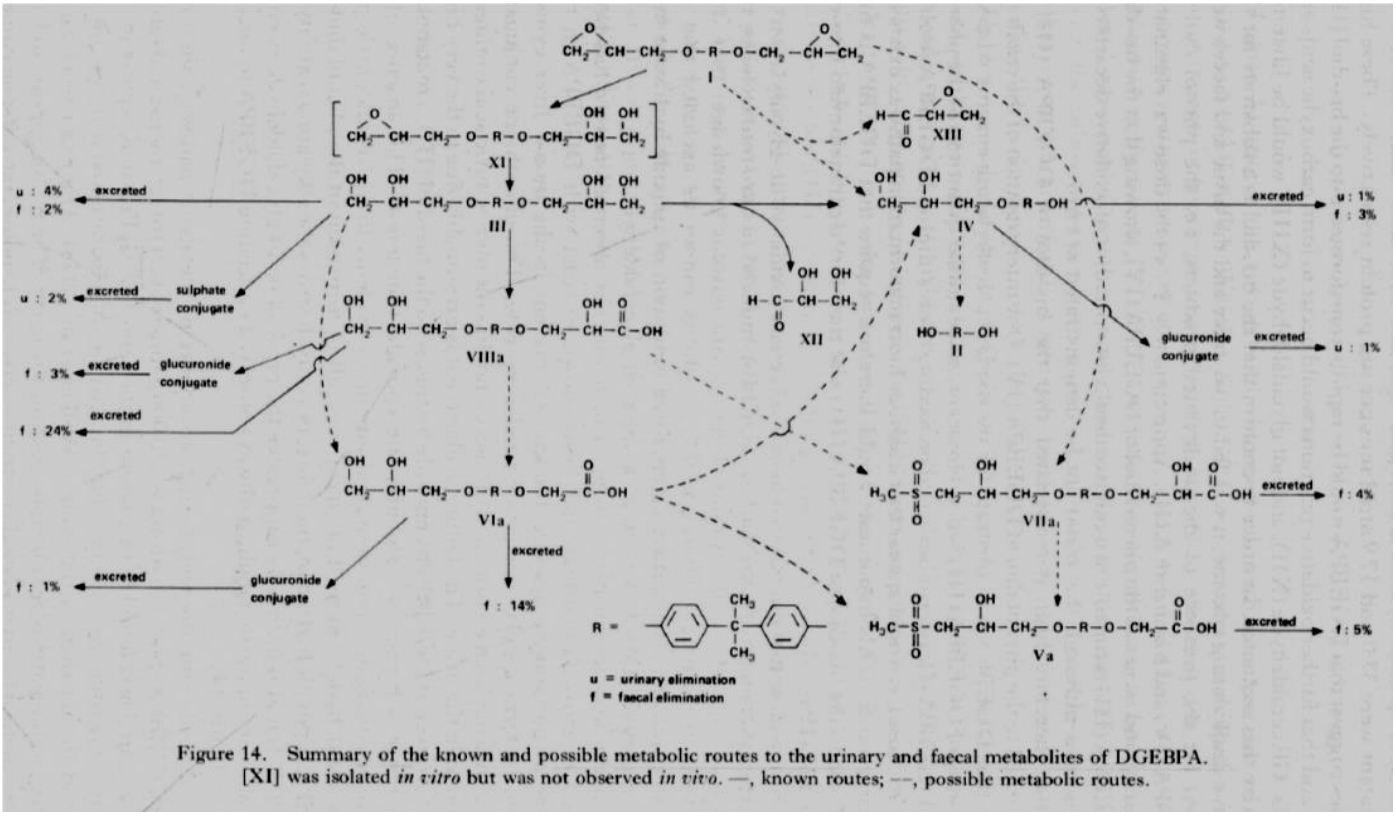


Figure 14. Summary of the known and possible metabolic routes to the urinary and faecal metabolites of DGEBPA. [XI] was isolated *in vitro* but was not observed *in vivo*. —, known routes; ---, possible metabolic routes.

Figure 1: Metabolism of BADGE from Climie et al. (1981b)

One of these carboxylic acids (VIIIa in Figure 1) resulted from direct biochemical oxidation of one of the diol groups in the bis-diol of BADGE (III in Figure 1) and was the major metabolite excreted in feces (27% of the administered dose).
Another of the carboxylic acids (VIa in Figure 1) was a result of oxidative cleavage of one of the diol groups in the bis-diol of BADGE (III in Figure 1), or of oxidative decarboxylation of 'VIIIa', and was also excreted in feces (15% of the administered dose).

Two unexpected carboxylic acids (VIIa and Va in Figure 1) were also identified in feces (4% and 5% of the administered dose, respectively). They contained a 3-methylsulphonyl-2-hydroxypropoxy side chain in place of the 2,3-dihydroxypropoxy side-chain on the opposite end of 'VIII' and 'VI', respectively. The origin of these sulfur-containing metabolites was uncertain and could possibly be oxidative artefacts from their respective glutathione conjugates; in Figure 1 these two metabolic routes are denoted as 'possible routes', i.e. not 'known routes'. The authors noted that the presence of these two metabolites is the only evidence in this study to show a possible direct alkylating reaction of BADGE, but as the extent of the reaction was very small compared to the other observed metabolic conversions, the authors considered that BADGE should not be considered as a highly reactive alkylating agent.

The phenol diol of BADGE (IV, near the top right in Figure 1) was identified in the urine and feces (5% of the dose). This metabolite could result by oxidative dealkylation of BADGE or of the bis-diol (III in Figure 1).

The three-carbon unit side-chain of BADGE could be released either as glyceraldehyde (XII in Figure 1), an endogeneous chemical, or as glycidaldehyde (XIII, top right in Figure 1), a known bacterial mutagen and mammalian skin carcinogen. Whether 'XII' or 'XIII' is released *in vivo* depends on the exact sequence of biochemical events. Oxidative dealkylation of BADGE by mouse liver microsomal mono-oxygenase *in vitro* leading to the formation of 'XIII' was not detected in this study; in Figure 1 this metabolic route is denoted as a 'possible route', i.e. not a 'known route'. The high activity of mouse liver BADGE epoxide hydratase suggests, according to the authors, that BADGE is rapidly hydrolysed and transformed to the bis-diol (III in Figure 1) *in vivo* and that further oxidative reactions would occur to form carboxylic acids and phenols as well as the release of glyceraldehyde (XII in Figure 1), whilst glycidaldehyde would not be formed (XIII in Figure 1).

In rats, different metabolites were excreted following oral and iv administration of BADGE. Less than 10% of the radioactivity in the plasma was identified as the parent compound (BADGE) following oral administration. This suggests that only a small percentage of the orally administered BADGE reached the systemic circulation unchanged. Hydrolysis of the epoxide groups of BADGE in the gastrointestinal tract could explain the poor absorption of orally administered BADGE and account for the observed differences in the fate of orally and iv administered BADGE. Studies on the stability of BADGE in simulated gastric fluid confirmed that over a third of the orally administered BADGE would be degraded in the gastrointestinal tract (unpublished report (1981a)).
The single study performed in rabbits indicated that a larger proportion of the bis-diol metabolite is excreted in the conjugated form in rabbit compared with the mouse; the total amount of the bis-diol metabolite excreted in the urine after a single oral dose of BADGE was about 10% of the dose following administration of 5 mg/kg bw and about 2% following 50 mg/kg bw (unpublished report (1983)).
In a report from the EU Commission (EC 2002) and in a review by Poole et al. (2004) it is mentioned that Coveney (1983) reported that metabolic pathways in the rabbit appeared similar to those in the mice (the reference 'Coveney (1983) is identical to the reference 'unpublished report (1983)', i.e. the rabbit study).

In the *in vitro* studies included by the registrant in the toxicokinetic section of the CSR (Boogaard et al. (2000b)), only a very limited chemical reaction with glutathione (GSH) was observed with BADGE in subcellular fractions of liver and lung of human, rat and mouse. BADGE was rapidly hydrolyzed by epoxide hydrolase (EH) to the corresponding bis-diol in cytosolic and microsomal fractions of liver and lung. The microsomal EH was more efficient that the cytosolic EH in hydrolysis of BADGE and the human microsomal fractions were more efficient in hydrolysis of BADGE than the rodent microsomal fractions at low substrate concentrations.

Another *in vitro* study (Bentley et al. 1989) was summarised in the report from the EU Commission (EC 2002) and in the review by Poole et al. (2004): The hydrolysis of the epoxide functionalities of BADGE by the microsomal and cytosolic fractions of mouse liver and skin

was investigated. It was reported that BADGE was rapidly hydrolyzed by EH of both tissues, with skin microsomal activity about 10 times higher than that found in the cytosol of skin. In other experiments using *in vitro* systems of liver fractions obtained from mouse, rat and rabbit the two epoxide groups of BADGE were very rapidly hydrolyzed to form the corresponding bis-diol. Further experiments showed no changes in the metabolism under conditions, which inhibited the EH activity or promoted other breakdown mechanisms (for example oxidative metabolism) (Bentley et al. 1989 as reported in Poole et al. 2004).

In humans, BADGE (no information on specification available) and three BADGE derivatives (bisphenol A bis (2,3- dihydroxypropyl) ether), i.e. the bis-diol of BADGE, bisphenol A (2,3-dihydroxypropyl) glycidyl ether, i.e. the mono-diol of BADGE, and bisphenol A (3-chloro-2-hydroxypropyl) (2,3- dihydroxypropyl) ether, - collectively referred to as BADGEs in the article) were found in all of the urine samples analyzed from US adults and from Chinese adults and children. Both free and conjugated forms of BADGEs were present in the urine. Among the four BADGE derivatives measured in urine, the bis-diol of BADGE was the predominant urinary metabolite with 45-60% of the total BADGEs concentration, followed by the unchanged BADGE found in 17-24% of the total BADGEs concentration (Wang et al. (2012). The eMSCA evaluates that presence of chlorinated metabolite indicates the presence of chlor in the substance, the persons were exposed to.

Similarly, in the study by Asimakopoulos et al. (2014), the bis-diol of BADGE (BADGE·2H_2O) was the predominant metabolite found in urine samples collected from Greek volunteers, accounting for about 90% of the five BADGEs measured in the urine. The other four BADGE-derivatives accounted for a total of about 35% of the five BADGE derivatives measured in urine (BADGE·H_2O HCl, 19%; BADGE·H_2O (the mono-diol of BADGE), 9%; BADGE, 4%; and BADGE·HCl, 3%). The authors noted that the distribution profile of the five BADGE derivatives found in human urine was similar to that reported in animal studies (species not specified), which confirmed that the primary product of BADGE metabolism is the bis-diol of BADGE. The authors also noted that the high detection rate of the bis-diol of BADGE in human urine also can result from direct intake via foodstuffs, as BADGE hydrolyses rapidly in water and that the bis-diol of BADGE has been reported to occur in canned foods. As above, the eMSCA considers that the occurrence of chlorinated metabolites may originated from the substance the volunteers were exposed to.

Based on the information in the described studies, potential species differences in the metabolism of BADGE between rats and other species, including humans, cannot be evaluated.

No information on the metabolism of DGEBA has been included by the registrant in the toxicokinetic section of the registration.

7.9.1.1.2.1. Possible formation of bisphenol A

In the comprehensive metabolism studies in mice given a single oral dose of BADGE (Climie et al. (1981b), unpublished report (1979a)), bisphenol A (BPA) was not detected in the urine or feces in mice in either the free or conjugated form. However, as oxidative dealkylation of the bis-diol (III in Figure 1) leading to the formation of the phenol diol of BADGE (IV in Figure 1) has been shown as a minor metabolic pathway in the mouse, the possibility of further oxidative dealkylation of the phenol diol of BADGE (IV in Figure 1) leading to the formation of BPA cannot be excluded. In Figure 1 formation of BPA (II, mid right in Figure 1) from the phenol diol of BADGE (IV in Figure 1 above) is denoted as a 'possible route', i.e. not a 'known route'.
In the report from the EU Commission (EC 2002) and in the review by Poole et al. (2004) it is mentioned that additional evidence indicating the inability of the mammalian biotransformation systems to convert BADGE to BPA was obtained in a similar experiment with BADGE diol, where BPA was not found in the urine or the feces.

The formation of BPA was not addressed in the *in vivo* studies in rats (unpublished report (1981a)) and rabbits (unpublished report (1983)), or in the *in vitro* study (Boogaard et al. (2000b)), i.e. the studies included by the registrant in the toxicokinetic section of the CSR.

Two other *in vitro* studies have been summarised in the report from the EU Commission (EC 2002) and in the review by Poole et al. (2004):
The metabolism of five different glycidyl ethers, including BADGE has been examined in human, rat and mouse liver, lung, and skin preparations (Boogaard et al. 2000a). The results of the metabolic studies found no evidence for any significant formation of BPA in incubations of BADGE with human or rodent liver or lung microsomal or cytosolic fractions, nor in incubations with intact viable human or rodent skin.
Formation of BPA from BADGE was not observed in any of the *in vitro* experiments performed by Bentley et al. (1989) who investigated the hydrolysis of the epoxide functionalities of BADGE by the microsomal and cytosolic fractions of mouse liver and skin.

The limited data on BPA formation in humans exposed to BADGE, i.e. the studies included by the registrant in the toxicokinetic section of the CSR (Hanaoka et al. (2002), Wang et al. (2012), Asimakopoulos et al. (2014)), are inconclusive.

A significant difference in BPA concentrations was observed between epoxy resin sprayers and the controls in the study the cross sectional by Hanaoka et al. (2002).
According to the registrant, the study used methods which were not acceptable and/or contained insufficient detail for an assessment to be made with expert judgement. Specifically, the study (1) did not use an authentic stable isotope of BPA; (2) the authors used background subtraction with a "blank sample" of urine (containing ~0.5pmol/ml). There were insufficient details on measured BPA levels to assess how this background subtraction related to the measured levels in the study samples; and (3) there was no data on BADGE concentrations in the subjects. The registrant also noted that the authors also failed to measure BADGE exposures and there was no mass balance to account for BADGE contributions to BPA levels. And that the authors did not consider other sources of BPA (aside from alcohol consumption and consumption of canned beverages). The eMSCA agrees with the criticism of the registrant.
The eMSCA also noted that the authors referred to the *in vivo* mouse metabolism study performed by Climie et al. (1981b) and mentioned, among other things, that BPA was one of the minor metabolites. However, this does not concur with the article by Climie et al. (1981b) or the original study report (unpublished report (1979a)) where BPA was not detected in the urine or faeces in either the free or conjugated form (see the study description above), i.e. Hanaoka et al. (2002) did not cite the mouse study correctly.
In conclusion, the Hanaoka et al. study conduct and reporting suffers from several limitations and the study is therefore not reliable. Consequently, there is no evidence of formation of BPA following exposure to BADGE in epoxy resin sprayers, based on the information available from this study.

Total BPA concentrations were also measured in the urine samples from humans in the study by Wang et al. (2012). It was shown that the concentration of BPA in the individual urine samples was 3- to 4-fold lower than the concentration of BADGEs. When correlating the concentrations in the range of the 0-95th percentiles of total BPA concentrations and total BADGEs concentrations, a significant linear relationship was observed ($r = 0.437$, $p < 0.0001$). According to the authors, this indicates that the sources of human exposure to BPA and BADGEs are related. However, the authors noted that earlier studies have reported the lack of transformation of BADGE to BPA *in vivo* in animals and *in vitro*, and therefore, formation of BPA from BADGE in humans is not likely, based on the currently available information.
The eMSCA agrees with the evaluation of the authors that formation of BPA from BADGE in humans is not likely, based on the currently available information in this study.

The formation of BPA was not addressed in the study by Asimakopoulos et al. (2014) in which total concentrations of BADGE and four derivatives were determined in human urine.

Acording to the report from the EU Commission (EC 2002) and the review by Poole et al. (2004), the available data demonstrate that mammalian metabolic systems are unable to transform BADGE into BPA and it can be concluded that human consumption of food containing low levels of BADGE will not lead to systemic exposure of BPA. The eMSCA agrees that formation of BPA from BADGE in humans is not likely, based on the currently available information from animal studies, as well as the limited information from the human studies. However, as oxidative dealkylation of the bis-diol of BADGE leading to the formation of the phenol diol of BADGE has been shown as a metabolic pathway in the mouse, the possibility of further oxidative dealkylation of the phenol diol of BADGE leading to the formation of BPA cannot be excluded.

7.9.1.1.2.2. Possible formation of a carcinogenic metabolite

The main metabolism of BADGE occurs by ring-opening of the two epoxide rings to form diols. This metabolite (the bis-diol of BADGE) is excreted in both free and conjugated forms and is further metabolized to various carboxylic acids. In the article by Climie and co-workers (1981) two possible routes of oxidative dealkylation of the glycidyl moiety of BADGE, through hydrolysis and through direct oxidation, were proposed (Figure 2).

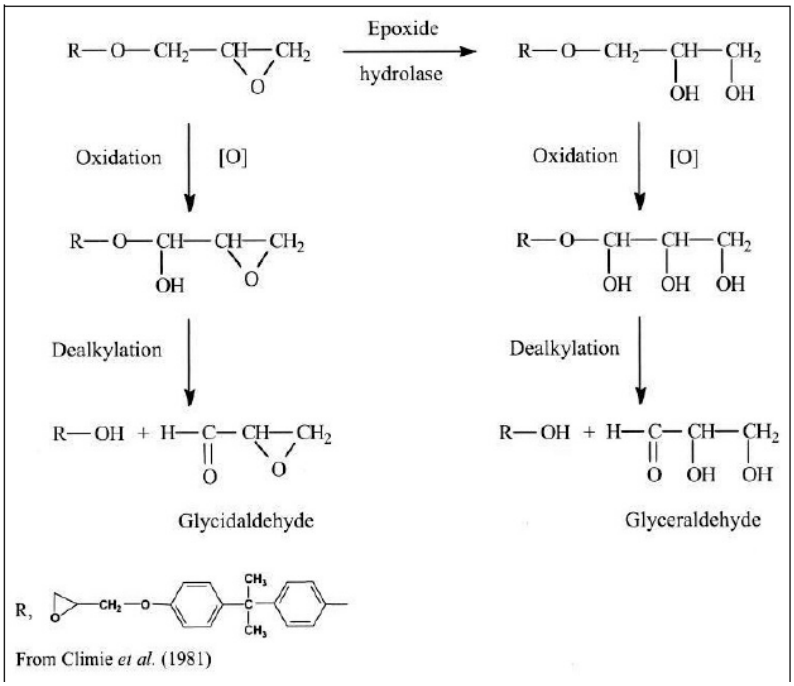


Figure 2: Two possible routes of oxidative dealkylation of the glycidyl moiety of bisphenol A diglycidyl ether (Climie et al, 1981b cited in IARC, 1999)

The two routes would lead to the possible formation of glyceraldehyde and glycidaldehyde, the latter being classified as a category 1B carcinogen by IARC. Although the formation of glycidaldehyde in humans cannot be completely excluded, the high activity of epoxide hydratase in relation to BADGE suggests that glyceraldehyde is the predominant metabolites in humans.

7.9.1.1.2.3. Conclusion on toxicokinetics

Following oral administration to mice, BADGE was rapidly and extensively excreted with the fecal route being the major route of elimination (approximately 80% of the administered dose) while a small fraction of the dose was recovered in the urine (approximately 10% of the administered dose). The percutaneous absorption of BADGE in mice was slow. The

recovery of radioactivity in organs and tissues was low both after oral and dermal administration, except for the skin following dermal application.
Similarly, BADGE was eliminated mainly by excretion via the feces following oral administration of BADGE to rats and the recovery of radioactivity in organs and tissues was also low in rats.

The metabolism of BADGE has been studied in mice, rats and rabbits, as well as in tissue fractions *in vitro*. Limited human information is also available.

In conclusion, the predominant metabolite of BADGE is the bis-diol of BADGE. In mice, this metabolite was further metabolized to various carboxylic acids. Formation of BPA from BADGE in humans is not likely, based on the currently available information. However, as oxidative dealkylation of the bis-diol of BADGE leading to the formation of the phenol diol of BADGE has been shown as a metabolic pathway in the mouse, the possibility of further oxidative dealkylation of the phenol diol of BADGE leading to the formation of BPA cannot be excluded. However, no BPA was found in the available metabolism studies in animals or humans.

A metabolic pathway including oxidation is possible leading to the possible formation of the carcinogenic substance glycidaldehyde. However, the high activity of epoxide hydratase suggests that hydrolysis is the predominant *in vivo* pathway, leading to the formation of glyceraldehyde and not glycidaldehyde.

Based on the available information on metabolism, potential species differences in the metabolism of BADGE between rats and other species, including humans, cannot be evaluated.

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

Not evaluated.

7.9.4. Repeated dose toxicity

This endpoint is not evaluated in full. However, results of experimental studies on repeated dose toxicity in which parameters relevant for reproductive toxicity and endocrine disruption were investigated are summarized below.

Summary and discussion of repeated dose toxicity

Several dietary and oral gavage subchronic (90 days) and sub-acute (28 days) studies have been performed with varying doses of BADGE. In these studies, the reproductive organs and endocrine sensitive organs were not examined, and these studies have therefore not been included in the table below. These are therefore only summarised below. The summary is from EU WRC report (EU 2002).

A study planned as a two-year oral gavage toxicity/oncogenicity study in Fischer 344e rats using 65 rats /dose level was initiated. The nominal doses were 0, 50, 250, and 1000 mg/kg bw/day. The animals were dosed 7 days per week. However, due to excessive toxicity in the two highest dose groups the study was terminated after 99 days (males) or 101 days (females). Oon a subset of 10 rats/sex/dose level toxicological parameters consistent with those required in a 90- day study (OECD TG 408) were evaluated.
Six animals died or were euthanized prior to study termination. Three high-dose rats, 2 males and one female, were euthanized in moribund condition, and showed moderate to severe acute tubular necrosis of the kidneys. A female at 50 mg/kg/day died on day 89.

Two males, one in the control and one in the high dose in groups were euthanized because of gavage-induced trauma to the upper esophagus of both animals.
Feed consumption and body weights were decreased throughout the study (BW decreases of 19.2% in males and 10.9% in females; 10.8% in males and 5.1% in females at top and mid-dose, respectively, whilst there was No effect on BW in males and a decrease in BW of 3.2% in females compared to controls.
At the high dose (1000 mg/kg bw/d) decreases in absolute (g) organ weights, and/or increases in relative organ weights were seen in males for all organs measured. Also, a statistically significantly higher mean relative testis weight was reported. Females at this dose level showed decreases in absolute adrenal gland, heart, ovaries, spleen and uterus weights, and increase in relative brain weight. At 250 mg/kg bw/day males showed decreases in absolute weight of the adrenal gland (15%), the heart (7%) and the spleen (10%), and increases in relative brain (8%) and testes weights (9%), whilst females had decreases in absolute adrenal gland and heart weights, and a statistically significant increase in relative brain weight. Treatment-related histopathologic effects in (surviving) animals given 250 and/or 1000 mg/kg/day were noted in the adrenal glands, cecum, ileum, kidneys, liver, testes, and uterus. (An industry consortium organized by the Epoxy resins committee of (2001)

In a sub-chronic dietary study, male rats were fed pure BADGE in their diets for three months at concentrations of 0, 0.1, 0.3, 1.0 or 3.0% (unpublished study from 1958 cited in EU, 2002). These percent values have been converted to 90,270, 900 or 2700 mg/kg/day by using the conversion factor 0.09 for sub-chronic studies suggested by EFSA (EFSA, 2012). Rats at the highest level (2700 mg/kg) rejected the diets and failed to gain weight; these rats showed effects upon gross and histopathological examination that were consistent with under-nutrition. Liver and kidney weights were higher as compared to controls, in the animals on the 3% diet. Animals in the 1% dose group exhibited slight enlargement of the kidneys with no adverse effects being seen in the 0.3 and 0.1% dose groups. No evidence of systemic toxicity, based on investigation of changes in haematology, clinical chemistry, gross pathology or histopathology of the spleen, heart, liver, kidney or adrenal glands, was found in any of the treated animals.

In another sub-chronic study a commercial low molecular weight BADGE epoxy resin (EPON 828) was fed to male Long-Evans rats at dietary concentrations of 0.2, 1.0 and 5.0 percent for 26 weeks (Hine *et al.* 1958). These percent values have been converted to 180, 900 or 4500 mg/kg/day by using the conversion factor 0.09 for sub-chronic studies suggested by EFSA (EFSA, 2012). All rats at the highest dose (4500 mg/kg/day) died by the end of 2 weeks, but gross and histopathological examination did not reveal evidence of systemic toxicity at any dose. There was no statistical increase in liver: body weight ratios, but there was a significant increase in kidney weights in groups fed 0.2 and 1.0% of EPON 828.

In an sub-acute oral toxicity GLP guideline study from 1984, (Sub-acute toxicity oral), 5 male and 5 female rats, (RA1f SPF), were treated, by oral gavage, with a commercial grade low molecular weight BADGE epoxy resin, (Araldite GY 250) for 28 consecutive days (unpublished study from 1984 cited in EU,2002). The doses used were 0, 50, 200 and 1000 mg kg/bw/day. These treatments had no effect on body weights, food and water consumption, food conversion, mortality, eye and hearing tests, haematology, blood chemistry, organ weights and pathology. There were no clinical symptoms and there were no compound-related macroscopic or histopathological changes of the spleen, heart, liver, kidney or adrenal gland. The no observable effect level (NOEL) was reported as 1000 mg kg/bw/day.

In the performed repeated dose toxicity study (OECD TG 408) from 2001 (cf. table 13 above), decreases in body weight and feed consumption were seen at the two highest doses of 250 and 1000 mg/kg bw/day. Some organ weights (absolute and relative) were also affected and this could be a treatment-related effect. Based on slight alteration in body weights and increased serum cholesterol in female rats at 50 mg/kg/day, a no-observed-effect level (NOEL) was not determined. The increased cholesterol was

interpreted by the study director to be non-adverse because rats are relatively resistant to the induction of hyperlipidema and atherosclerosis even when serum cholesterol levels are elevated. The eMSCA finds that the cholesterol increases do raise some concern, as humans may be more sensitive to increased serum cholesterol levels than rats. Endocrine organs and hormone sensitive tissues were affected at 1000 mg/kg bw/day, with degeneration of the seminiferous tubules in the testes and atrophy of the endometrium of the uterus. The NOAEL for responses was for this study 250 mg kg body weight/day.

The OECD test guideline (TG) 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents) has been updated in 2018 with several endocrine endpoints after this study was performed. In the update the measurement of T4, T3, TSH and thyroid gland weight is required. In addition, serum total cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) should also be determined as levels of these parameters are directly controlled by thyroid hormone action and contribute (with other thyroid endpoints) to evidence of thyroid effects. Optional endpoints include other hormone measurements, as well as assessments of sperm parameters. Negative existing *in vivo* effects data from studies performed prior to the TG update should be interpreted with caution as the negative results may reflect that relevant endocrine end-points (such as thyroid hormone levels) were not investigated, or that the tests used do not have sufficient power to detect weak effects (OECD GD 150, 2018).

Even though a number of longer-term repeated dose studies are available in rodents, none of them clarifying the concern regarding possible endocrine disrupting effects of BADGE. This is because there is a lack of information on e.g. hormone levels that are not available from the older repeated dose toxicity studies. Moreover, the repeated dose studies do not evaluate effects in developing animals (such as the reproductive toxicity studies) and do not cover the most sensitive life stages for endocrine active compounds.

7.9.5. Mutagenicity

7.9.5.1 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 (unpublished reports 1981b, 1978b, 1977; Sueiro et al., 2001; Canter et al., 1986; Andersen et al., 1978). Two studies (both reliable (evaluating MSCA: Klimisch score 2)) including one found in the open literature have shown that DGEBA and BADGE caused gene mutations in the E. Coli strains WP2uvrA and IC3327 (Hemminki et al., 1980, 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 (eMSCA: Reliable (Klimisch score 2)) have yielded positive results for DGEBA (positive with and without metabolic activation) (unpublished reports 1982, 1989a). 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 (eMSCA: Reliable (Klimisch score 2)) (unpublished report 1981a).

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 (eMSCA: Reliable (Klimisch score 2))(Pfeiffer et al., 2000).

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 (Suarez et al., 2000).

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 (Hemminki et a., 1980).

***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. (eMSCA: Reliable (Klimisch score 2)) (unpublished report 1979b).

7.9.5.2 *In vivo* studies on somatic cells

Chromosomal aberrations *in vivo*

Several 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 (unpublished reports 1989b, 1978e, 1977).

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 BADGEgroups compared to the negative control (eMSCA: Reliable (Klimisch score 2))(unpublished study report 1989b).

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 (eMSCA: Reliable (Klimisch score 2)) (unpublished report 1978d).

Another chromosome aberration study (micronucleus test in bone marrow of Chinese hamsters (male/female)) was mentioned in the registration without a clear reference. It was conducted with sample size of 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) (unpublished report 1981c).

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 (Bentley et al., 1989).

7.9.5.3 *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. BADGE 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 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) (Unpublished report 1982a).

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 (Unpublished report 1982b).

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 (unpublished report 1977).

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.

Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay

In order to address the request in the substance evaluation decision of 17 May 2017 on mutagenic properties of BADGE, the registrant(s) chose to perform a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD TG 488 ver. 2013) (unpublished report, 2018). As requested, the study was conducted using a 28 + 3 day testing strategy and glandular stomach, duodenum and liver samples were analyzed for mutations.

The study was conducted in Transgenic Big Blue® Fischer 344 (F344) rats (5-6 animals pr. group) by assessing the mutant frequency of the *cII* gene. The study was conducted according to OECD 488 (2013) in accordance with US EPA GLP Standards 40 CFR 792 (TSCA).

The animals were dosed with BADGE in vehicle (0.5% Methocel A4M methyl cellulose ethers with 0.1% Tween 80 in deionized water) once per day on each of 28 consecutive days and sacrificed 3 days after the final administration. A dose volume of up to 10 mL/kg bw was used. Dose concentrations were 0, 250, 500 or 1000 mg/kg bw per day and Ethylnitrosourea (ENU) was used as positive control. All doses were administered at a volume of 10 mL/kg bw. A procedure of stirring and homogenising the test formulation was followed. After sonication, the formulations were heated between 35-45°C, while stirring. BADGE formulations where kept at 37 degrees Celsius and remade every 3 days. Test formulations for BADGE were stirred prior to and during dosing.

During the first week of the study it became apparent that the high dose animals displayed signs of excessive toxicity (hunched posture, moderate to slight diarrhea, squinty eyes, and ruffled fur as well as a low mean bodyweight). The reason for this is unknown, but it was speculated by the authors that the observed toxicity was caused by exaggerated high dose on days 6-7 possibly related to the lack of adequate formulation homogeneity, and/or in combination with a shorter post-dosing recovery period (morning dosing on day 6 vs. afternoon dosing previously i.e. 16 instead of 24 hrs between doses). Due to concerns about unknown effects on mutagenicity caused by potential excessive dosing, it was decided to terminate the high dose group on day 25 and discard the animals without tissue collection. The study was then extended to include an additional phase (extended phase) with animals from the same breeding group and three dose 0 mg/kg bw/day, 500 mg/kg bw/day, and 1000 mg/kg bw/day), otherwise following the same study design as in the initial phase.
During the extended phase, signs of toxicity in the 1000 mg/kg bw/day group was observed during cage-side or unscheduled observations and included decreased motor activity, ruffled fur, hunched posture, and squinty eyes, starting from day 9. All animals appeared normal after Day 12. There were no remarkable clinical observations associated with BADGE treatment at doses ≤500 mg/kg bw/day in either the initial or the extended phase.

Although the authors of the study speculated if lack of adequate formulation homogeneity could be a contributing factor to assessive toxicity ((hunched posture, moderate to slight diarrhea, squinty eyes, and ruffled fur weight loss) in the initial phase, homogeneity testing

was performed twice on the first days of concentration verification (CV1 and CV2) for the initial phase and for the first day of the third concentration verification at the beginning of the extended phase (CV3). It is unclear if test formulation was sonicated again prior to dosing or only stirred, which could hamper the homogeneity of the test substance thus impeding on the result of the study. No analysis of homogeneity was conducted around the time the incidence occurred, and therefore this suspicion can neither be confirmed nor rejected.

Although not as severe, signs of toxicity were also observed in the high dose animals in the extended phase of the study. The observed toxicity is an indication that the test material was systemically available to the animals during the study which decreases the concern that the test substance was not bioavailable due to insufficient homogenization.

The eMSCA noted that there were some discrepancies in the test performance related to the requirement stipulated in the final decision of 17 May 2017. Notably, it was stated in the decision that the preparation of the test formulation should be freshly made daily in an appropriate vehicle to ensure maximal exposure to unreacted BADGE, and that the choice of vehicle should be justified. These requirements were not followed and the test formulation was only prepared every third day. However, chromatograply stability data made available to the eMCSA substantiates that the substance is stable for at least three days at 37 degrees. A Klimish score of 2 was attributed by the eMCSA.

Determination of mutant frequency:

Mutant frequency (MF) is determined by dividing the number of plaques/plasmids containing mutations in the transgene by the total number of plaques/plasmids recovered from the same DNA sample. No statistically significant increase in MF was observed in any of the test groups for any of the tissues when compared to the concurrent vehicle.

MF of all individual control animals and BADGE treated animals for all tissues were within the 95% Control Limits (CL) of the historical background MF.

Liver		
Group	**Dose levels (mg/kg/day)**	**Mean MF ± Standard Deviation (x 10^{-6})**
Initial phase		
Group 1 (vehicle control)	0	25.1 ± 7.7 (median 26.5)
Group 2	50	24.7 ± 7.2
Group 3	250	24.0 ± 6.3
Group 4	1000	Not analyzed
Group 5 (positive control)	20 (days 1,2,3,12,19,26)	239.1**## ± 65.2
Extended phase		
Group 6	0	36.2 ± 5.2 (median 3.6*)
Group 7	500	25.0# ± 3.8
Group 8	1000	34.9 ± 10.0

* = Statistically significant (Kruskal-Wallis test, p = 0.028) compared to Group 1.
** = Statistically significant (1-Way ANOVA, p < 0.001) compared to Group 1.
# = Statistically significant (1-Way ANOVA, p = 0.027) compared to Group 6.
= Statistically significant (1-Way ANOVA, p < 0.001) compared to Group 6.

Duodenum		
Group	**Dose levels (mg/kg/day)**	**Mean MF ± Standard Deviation (x 10^{-6})**
Initial phase		
Group 1 (vehicle control)	0	43.3± 18.8 (median 40.0)
Group 2	50	40.2 ± 20.1
Group 3	250	29.3 ± 9.6
Group 4	1000	Not analyzed
Group 5 (positive control)	20 (days 1,2,3,12,19,26)	840.9** ± 85.8 (median 826.0##)

Extended phase		
Group 6	0	34.6± 12.4 (median 34.2)
Group 7	500	26.4 ± 8.9
Group 8	1000	46.5 ± 12.0

** = Statistically significant (1-Way ANOVA, p < 0.001) compared to Group 1.
= Statistically significant (Kruskal-Wallis, p = 0.009) compared to Group 6.

Glandular stomach		
Group	**Dose levels (mg/kg/day)**	**Mean MF ± Standard Deviation (x 10^{-6})**
Initial phase		
Group 1 (vehicle control)	0	24.9 ± 4.5
Group 2	50	19.1 ± 3.5
Group 3	250	23.4 ± 6.9
Group 4	1000	Not analyzed
Group 5 (positive control)	20 (days 1,2,3,12,19,26)	512.7**## ± 77.6
Extended phase		
Group 6	0	31.2 ± 9.3
Group 7	500	19.7 ± 0.9
Group 8	1000	28.5 ± 10.9

** = Statistically significant (1-Way ANOVA, p < 0.001) compared to Group 1.
= Statistically significant (1-Way ANOVA, p < 0.001) compared to Group 6.

In conclusion, the performed Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay in glandular stomach, duodenum and liver yielded a negative result in all somatic tissues tested when mice were exposed to BADGE by oral gavage at concentrations up to 1000 mg/kg bw/day.

7.9.5.4 Conclusion on Mutagenicity

BADGE has yielded positive results for gene mutations *in vitro* in the Ames test both with and without metabolic activation. 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 a micronucleus test without metabolic activation. BADGE 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*.

Two reliable *in vivo* studies (Klim 2.) which investigated chromosomal aberrations in somatic cells (bone marrow) yielded negative results. Chromosomal aberrations in mouse spermatocytes was addressed in two other studies that yielded negative/equivocal results. Although the results of these studies are inconclusive due to the inadequate study protocols used, one of these studies using a high dosage for 10 days duration indicated that BADGE is not likely to cause chromosomal aberrations under the 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.

As requested in the substance evaluation decision of 17 May 2017, the concern for genotoxicity at the initital side of contact was followed up with the performance of a Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD TG 488 ver. 2013) carried out in 2018. The eMSCA noted some discrepancies in the test performance compared to the requirement stipulated in the final decision as to the preparation of the test formulation. However performed analyses confirmed sufficient stability and homogeneity of the substance during the test, and a Klimish score of 2 was attributed. In

this study, BADGE did not increase the mutant frequency in glandular stomach, duodenum or liver samples under the conditions of the study.

Overall, the eMSCA concludes that BADGE is not mutagenic. The concern for mutagenicity of BADGE included in CoRAP has thus been clarified, and no further testing for this end-point is regarded to be necessary.

7.9.6. Carcinogenicity

No human data on the carcinogenicity of BADGE or the formerly registrered substance DGEBA are available.

The carcinogenicity studies in rodents included in the registration dossier appear to have been conducted with the formerly registered substance, bisphenol A diglycidyl ether, DGEBA or the presently registered substance, BADGE (1654-75-3). However, the test material was inadequately described in a number of studies. Only a limited number of animal studies exists using pure (analytical grade) BADGE, with most studies using technical grade material, containing various impurities.

Carcinogenicity: oral

Table 14. Study on carcinogenicity after oral administration

Method	Results	Remarks	Reference
Combined chronic/carcinogenicity study rat (Fischer 344) male/female oral: gavage 0, 2, 15, or 100 mg Bisphenol A Diglycidyl Ether (BADGE)/kg/day (nominal conc.) Exposure: up to 24 months (daily) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	Histopathological examination of survivors from the low and intermediate dose levels was limited to the liver, kidneys, lungs, spleen, and relevant gross lesions. Males given 15 or 100 mg/kg/day had treatment-related statistically significant decreases in body weights and body weight gains. After one year of dosing, body weight gains for males given 15 or 100 mg/kg/day were 4.0% and 12.9% lower than controls, respectively. At study termination body weights of the males given 15 or 100 mg/kg/day were 3.5% and 7.4% lower than controls, respectively. There were no treatment-related effects on body weights of males given 2 mg/kg/ day nor of females from any dose group. NOAEL (toxicity): 15 mg/kg bw/day (nominal) (male) (decreased body weight and cecum enlargement) NOAEL (toxicity): 100 mg/kg bw/day (nominal) (female) (cecum enlargement) NOEL (toxicity): 2 mg/kg bw/day (nominal) (male/female) No increase in neoplasms was observed in either male or female rats at any dose level. The authors of the study conclude that bisphenol A diglycidyl ether did not show oncogenic potential under the conditions of this study.	reliable key study Test material (EC name) reported by registrant as: 4,4'-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane, the name previously used in the registration dossier. However the corresponding CAS RN 25068-38-6 is not reported in robust study summary. Test material reported in NL report to be BADGE purity for >99%)	Unpublished report (2004) Only RSS available to eMSCA Study summarised din NL Health Council (2013)

In the most recently conducted oral study, *(see table* 14 above), decreased body weight and an enlarged cecum were observed in male rats at 15 mg/kg/day in the oral study and

a NOAEL of 15 and 100 mg/kg/day in male and female rats, respectively was set. No oncogenic potential in the gastrointestinal tract or in other tissues were found.

Another feeding long-term/carcinogenicity study included in the registration is from 1958. The identity or purity of the the test substance is not clear. No NOAEL was identified as the lowest concentration in the feed administrated of 0.2% of the test material led to increased kidney weights. No neoplastic effects were seen. No tumours were reported. (Hine et al. 1958d – only RSS available to eMSCA).

Carcinogenicity: inhalation

No carcinogenicity studies on inhalation exposure to BADGE were available to the eMSCA.

Carcinogenicity: dermal

The results of studies on carcinogenicity after dermal administration are summarised in the following table:

Table 15. Studies on carcinogenicity after dermal administration

Method	Results	Remarks	Reference
Combined chronic/carcinogenicity study rat (Fischer 344) female 0, 1, 100 or 1000 mg BADGE/kg body weight/application (mka) (nominal conc.) Vehicle: acetone Exposure: dermal up to 2 years (5 times/week) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOEL (toxicity): 100 mg/kg/d (male/female) (Systemic effect on liver) Under the conditions of this 2-year dermal oncogenicity bioassay, bisphenol A diglycidyl ether at doses up to 1,000 mg/kg/application did not cause neoplasia in any tissue in female Fischer 344 rats.	1 (reliable without restriction) key study Test material (EC name) reported in RSS: 4,4'-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane CAS RN 25068-38-6, the former identity of the registred substance (purity unknown)	Unpublished study 1998a Only RSS available to eMSCA
Mouse (B6C3F1) male 0, 0.1, 10 or 100 mg BADGE/kg body weight/application (mka) (nominal conc.) Vehicle: acetone Exposure: dermal up to 2 years (3 times/week) OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	Dermal application of BADGE at doses up to 100 mg/kg/application for two years produced neither systemic toxicity nor oncogenicity in any tissue in the male B6C3F1 mouse. The no-observed-effect level (NOEL) for systemic toxicity was 100 mg/kg/application. The no-observed-effect level (NOEL) for dermal effects was 0.1 mg/kg/application.	reliable key study experimental result Test material (EC name) declared by registrants as: 4,4'-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane, DGEBA CAS RN 25068-38-6, the formerly identity of the registrered substance (purity unknown)	Unpublished study 1998b Only RSS available to eMSCA

The eMSCA notes that only a very small amount of bisphenol A diglycidyl ether is able to penetrate human, mouse and rat skin unchanged and that is metabolized to its corresponding –diol. Thus, only negligible amount of BADGE is expected to become systemically available after dermal application (Climie 1981a).

A number of dermal studies have been performed on mice with either pure ('analytical grade') bisphenol A diglycidyl ether or technical grades of bisphenol A diglycidyl ether. Pure ('analytical grade') bisphenol A diglycidyl ether was tested in one experiment by skin application in CF1 mice; no epidermal, but a few dermal tumours were observed in males and there was a small increase in the incidence of lymphoreticular/haematopoietic tumours in females (IARC, 1989).
IARC (1999) reported that C57BL/6 and CF1 mice treated with technical grade BADGE showed increased incidences of epidermal tumours. However, the effect was not reproduced in another study with CF1 mice. C3H mice did not show skin tumours in 2 studies. Small increases in the incidences of lymphoreticular/haemopoietic tumours were seen in CF1 mice treated with technical grade BADGE (IARC, 1999). Subcutaneous injection of technical grade BADGE to male Long-Evans rats entailed, a small number of local fibrosarcomas. No skin tumours were seen in albino rabbits treated dermally with technical grade BADGE.
In a dermal study with analytical grade BADGE in CF1 mice a few dermal tumours in males and a small increase in the incidence of lymphoreticular/haematopoietic tumours in females were observed, (IARC 1999).

In an unpublished study on dermal chronic toxicity/carcinogenicity of BADGE from 1998 included in the registration dossier (table 15 above), male B6C3F1 mice and female Fischer 344 rats were used (unpublished reports 1998a and 1998b). In the rats, a systemic NOEL of 1 mg/kg/day was set based on non-oncogenic histopathologic changes observed in the liver of female rats administered 10 and 100 mg/kg/day. The test material was not occluded and the changes were attributed to ingestion, as the dermal uptake of BADGE is low. In the mice, the NOEL at the application site was 0.1 mg/kg/day based on skin effects as epidermal hyperplasia, chronic dermal inflammation and epidermal crusts observed histopathologically at dosages of 10 and 100 mg/kg/application in male mice. However, no progression to skin tumours were reported. No oncogenic or systemic effects were reported in male mice, leading to a $NOAEL_{systemic}$ setting at 100 mg/kg/day A large dosing interval, and a relatively low high dose is noted in the mice dermal chronic/carcinogenicity study.

In relation to carcinogenicity of the possible BADGE metabolite glyceraldehyde no experimental or human data regarding carcinogenicity are found in the literature. The less likely metabolite glycidaldehyde is classified by IARC as possibly carcinogenic to humans (Category 2B) on the basis of sufficient evidence in experimental animals (IARC 1976, 1987, 1999): glycidaldehyde is carcinogenic in mice by skin application and by subcutaneous injection in rats. It produced malignant tumours at the site of application in both species. No epidemiological data relevant to the carcinogenicity of glycidaldehyde are available.

IARC has concluded that bisphenol A diglycidyl ether is not classifiable as to its carcinogenicity to humans (Group 3), based on lack of human data and limited evidence of carcinogenicity in animals on the substance (IARC, 1987). This conclusion was confirmed in 1999 (IARC, 1999).

The Health Council of the Netherlands) has also analysed the available information on carcinogenicity of BADGE. The committee appears to have had access to the same database as IARC in 1999. The Dutch report stresses that data are insufficient to conclude overall on the possible carcinogenicity of BADGE as the committee finds that information is lacking.
The committee acknowledges that pure (analytical) BADGE is not oncogenic in a number of animal studies. Dermal application of technical grades of BADGE were reported to occasionally increases in various tumours (slight increases in epidermal and kidney

tumours in male mice, and slight increases in the incidence of lymphoreticular/ haematopoietic tumours and reticulum cell sarcoma in female mice). However, the committee stresses that the low incidences and the lack clear, reproducible target organ reduces the concern for a relationship with exposure to BADGE. The Dutch report further points to a possible role of epichlorohydrin, that may be have been present in the technical grades used (Health Council of the Netherlands, 2013),

Based on this information the eMSCA evaluates that the concern for carcinogenicity is clarified, and that BADGE is not carcinogenic.

Conclusion on Carcinogenicity:

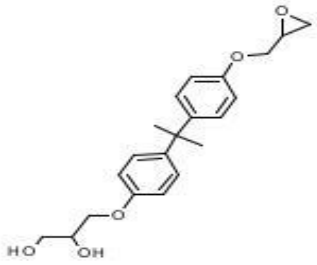
BADGE was concluded not to be mutagenic, and genotoxic carcinogenicity can be excluded.

Based on the overall available database on BADGE as now registered, the eMSCA considers that BADGE is not carcinogenic.

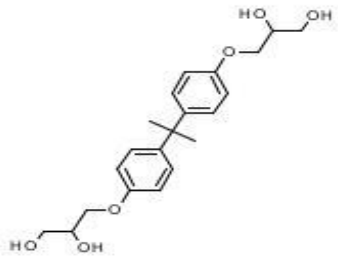
7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

(Q)SAR Reprotoxic and developmental effects

Predictions for BADGE and its main degradation products BADGE-mono-diol, and BADGE-bis-diol, with the chemical structures as shown below from left to right, respectively were predicted:



Badge_1906_7



Badge_1906_8

#	Substance	Lipinski	G.I. abs %
1	BADGE	Passed	99
7	BADGE-*mono*-diol	Passed	93
8	BADGE-*bis*-diol	Passed	84

Predictions obtained in models in the commercial MultiCASE CASE Ultra suite for mammalian reproductive toxicity ("Mammalian ReproTox Models"[2]) were negative, inconclusive or out-of-domain.

[2] Details on MultiCASE CASE Ultra Mammalian ReproToxModels:
A48 (Developmental toxicants, human) Model Version: 1.5.2.0.119.500
A49 - Teratogenic potential in humans
RP_AN1 (Female fertility, rodent) Model Version: 1.5.2.0.960.500
RP_AN5 (Female fertility, rat) Model Version: 1.5.2.0.895.500
RP_AN9 (Female fertility, mouse) Model Version: 1.5.2.0.151.500

Negative or indeterminate predictions were obtained in all models in Leadscope commercial Non-human Developmental and Reproductive Toxicity Suites[3], except for positive predictions for BADGE, BADGE-mono-diol and BADGE-diol for fetal weight decrease in rodents (wt dec rodent (A1)). Positive predictions for weight decrease in rats in another model (weight dec. rat) for BADGE-mono-diol and BADGE-bis-diol. If a probability cut-off of 0.6 instead of 0.7 for positives is applied (default by the US FDA), then also the registered substance BADGE is positive for weight decrease in rats.

In a small commercial MultiCASE CASE Ultra model for developmental human toxicity (A48) negative predictions were obtained for BADGE-bis-diol and in the MultiCASE commercial CASE Ultra model for human teratogenicity potential (A49), whilst the BADGE and BADGE-mono-diol were out of domain.

7.9.7.1 Effects on fertility *in vivo*

Results of experimental studies investigating effects on fertility relevant for the end-point of concern on endocrine disruption are summarized in the table below.

RP_AO1 (Male fertility, rodent) Model Version: 1.5.2.0.784.500
RP_AO4 (Male fertility, rat) Model Version: 1.5.2.0.715.500
RP_AO7 (Male fertility, mice) Model Version: 1.5.2.0.146.500
RP_AP1 (Sperm toxicity, rodent) Model Version: 1.5.2.0.906.500
RP_AP4 (Sperm toxicity, rat) Model Version: 1.5.2.0.722.500
RP_AP7 (Sperm toxicity, mouse) Model Version: 1.5.2.0.262.500
RP_AQ1 (Newborn behavioral toxicity, rodent) Model Version: 1.5.2.0.666.500
RP_AQ4 (Newborn behavioral toxicity, rat) Model Version: 1.5.2.0.622.500
RP_AQ9 (Newborn behavioral toxicity, mice) Model Version: 1.5.2.0.173.500

[3] Details on Leadscope commercial Non-human Developmental and Reproductive Toxicity Suites:

Growth Retard Mouse	Pre Impl Loss Rodent
Growth Retard Rabbit	struct mouse (AL6)
Growth Retard Rat	Dysmorph Rabbit
retard rodent (AH1)	Dysmorph Rat
Wt Dec Mouse	struct rodent (AL1)
Wt Dec Rabbit	Visc Org Mouse
wt dec rodent (AI1)	Visc Org Rat
weight dec. rat	Visc Org Rodent
Fetal Death Mouse	Repro Mouse Female
Fetal Death Rabbit	Repro Rat Female
Fetal Death Rat	Repro Rodent Female
Fetal Death Rodent	Repro Mouse Male
post impl mouse (AG6)	Repro Rat Male
Post Impl Loss Rabbit	Repro Rodent Male
Post Impl Loss Rat	sperm mouse(AP5)
post impl rodent (AG1)	Sperm Eff Rat
Pre Impl Loss Mouse	Sperm Eff Rodent
Pre Impl Loss Rabbit	
Pre Impl Loss Rat	

Table 16 Overview of experimental studies on fertility

Method	Results	Remarks	Reference
One-generation study oral: gavage **OECD Guideline 415** (One-Generation Reproduction Toxicity Study) (**1981**). Rat (CrL:CD(SD) BR VAF/Plus) male/female 0, 20, 30, 180, 540 mg/kg/day (nominal conc.), analysed concentration: 13.1-17.4 mg/kg/day, 50.6-55.0 mg/kg/day, 154.8-166.3 mg/kg/day and 464.4-499.0 mg/kg/day respectively for nominal doses of **20, 60, 180 and 540** mg/kg/day, respectively. Thus values were 8-35% below of that intended). Vehicle:0.5% carboxymethylcellulose and 0.1% (v/v) Tween 80. Exposure: Males dosed 10 weeks prior to mating, through mating to termination (PND 21). Sexually mature females treated daily for two weeks prior to mating until weaning of F1 offspring (PND 21). Organ weight analysis was performed on F0 adults and reproductive tract of all animals, and alimentary tract of control and high dose group animals (540 mg/kg/day) was examined histologically.	No statistically significant adverse effects were observed on sexual function or fertility end-points including mating performance, gestation period, mean pre-birth or total litter loss, or on the ability of females to rear their offspring successfully to weaning were observed at any exposure dose (up to 540 mg kg body weight/day). Slightly reduced food consumption for females and a lower mean body weight in males at 540 mg/kg/day during the first week of treatment. No treatment-related macroscopic changes, differences in mean organ weights or histological changes to the reproductive tract (and alimentary tract) (only assessed in top dose) in either sex of the F0 generation were observed. Postnatal development is presented in *Table 17.*	1 (reliable without restriction) / OECD Guideline 415 (One-Generation Reproduction Toxicity Study) Test material: 4,4'-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane (DGEBA), (CAS RN 25068-38-6), content of BADGE, CAS RN 1675-54-3) = 79.7%. Impurities not identified	Unpublished report(1989)
Two-generation study Rat (Sprague-Dawley) male/female 30 male and 30 females per group Oral gavage 0, 50, 180, 540 or 750 mg/kg/day (nominal conc.). Vehicle: aqueous solution of 0.5% Methocel A4M (Trademark, Dow Chemical) with 0.1% Tween 80 Exposure: 238 days total (once daily). OECD Guideline 416 (Two- Generation Reproduction Toxicity Study) (1996). Parameters evaluated over the course of the study included body weights, feed consumption, clinical observations, mating performance, and gross pathology and histo-pathology of the adults, as well as neonatal growth and survival of the offspring.	Mating and conception indices for both males and females were lower than normal in the P generation, but the lowest conception values were found in the control group, where only 50-60% of the females produced litters. The largest numbers of litters were found in the highest dose group, where fertility indices in females ranged from 73% to 93%. There were no statistically significant treatment-related effects on reproductive performance. Postnatal development is presented in *Table 17 .*	1 (reliable without restriction) key study Test material BADGE, CAS RN 1675-54-3, purity of 99.65%	Unpublished reports (1996 and 1997)

Method	Results	Remarks	Reference
Acute toxicity of BADGE on testis of 5 male SPF Sprague-Dawley rats / group. **Single dose** of 500, 750, 1000, and 2000 mg/kg/day administered by gastric lavage. The right testis was processed for light microscopic analysis. The left testis was homogenized and spermatids were counted to determine the daily sperm production and daily abnormal sperm production. The sperm count, sperm motility, and incidence of abnormal sperm were estimated in the epididymis. The progression of spermatogenesis was arbitrarily classified as full-matured, maturing, and immature.	750, 1000, and 2000 mg/kg/day: increased number of immature and maturing sperm in the testis. No significant differences with respect to sperm head count, sperm motility, and sperm abnormality in any treatment group. Conclusions from study authors: These results suggest that single oral exposure of BADGE 750 mg/kg/day can affect adult male testis development.	Reliability: weak No explanation for study design (only one single dosing point). No Purity of BADGE is provided in this study. The data from this study are not particularly robust, for the evaluation of potential *in vivo* effects of BADGE on testis.	Yang et al. (2010)

Summary and discussion of studies investigating effects on fertility

Two guideline studies on fertility in rats, a one-generation study (OECD TG 415) from 1989 and a two-generation study (OECD TG 416) from 1996, are available in the registration dossier. Moreover, a non-guideline study of toxicity to the testis following a single exposure to BADGE to male rat was also performed. The studies are described in table 16 above and also in brief below.
In the performed one generation assay (OECD TG 415) no statistically significant adverse effects were observed on sexual function or fertility end-points. The one-generation assay has now been deleted by OECD (December 2019) as it has been made redundant following the introduction of the Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) in2018.

In the two generation study, administration of BADGE (as DGEBPA) at dose levels up to 750 mg/kg bw/day resulted in only slight toxicity. Among adult males, body weights were decreased approximately 8 -11% at dose levels of 540 and 750 mg/ kg/day in both the P1 and P2 generations (TG 416), although there was no statistically significant decrease in the body weights of the P1 males treated with 750 mg/kg/day until test day 238 (i.e. termination).

In females, body weights were also affected in both generations (TG 416), but only at the highest dose level (750 mg/ kg/day). Secondary changes in absolute and/or relative liver and kidney weights were also observed in these dose groups. There were no treatment-related effects on body weights among males given 50 mg/kg/day, or among females given 540 or 50 mg/kg/day in either generation. There were no treatment-related histologic changes noted in any dose group.

BADGE displayed no indications of any adverse effects on fertility in rats over two generations at any dose levels. The NOAEL for adult males was considered to be 50 mg/kg/day, NOAEL for adult females 540 mg/kg/day, and the NOAEL for reproductive effects was 750 mg/kg/day, the highest dose tested.

The OECD two-generation and one-generation reproduction toxicity studies are apical assays designed to provide general information concerning the effects of a chemical on the male and female reproductive systems including gonadal function, the oestrus cycle, mating, conception, gestation, parturition, lactation, weaning and growth and development of the offspring. The studies are designed to provide data on adverse effects on

reproduction and development. The studies are not specifically designed to detect endocrine disruptors but they include some endpoints relevant for the assessment of possible endocrine disruption. However, the one-generation study (OECD TG 415), adopted in 1983, only includes one cycle of mating and is much less prescriptive in both the performance of the study and the endpoints to be assessed. The TG has now been deleted (as of dec. 2019 as mentioned above). In contrast, the two-generation study (OECD TG 416) includes two cycles of mating and the original OECD TG 416 was revised in 2001 to include a more comprehensive range of endpoints. These endpoints include sexual maturation (VO and PPS) which are particularly sensitive to EDs. One-generation and two-generation studies conducted prior to the adoption of the revised OECD TG 416, like in this case with BADGE, are therefore unlikely to provide as much data as studies conducted to the revised OECD TG 416, particularly with respect to endocrine disruption as sexual maturation, estrous cycle and semen parameters, but not serum hormone measures (OECD 2018) (OECD, 2012a). Among the current OECD, test guidelines for mammalian reproductive toxicity, exposure during all vulnerable periods of development is performed in the Extended
One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) and the
Two-Generation Reproductive Toxicity Study design (OECD TG 416). The EOGRTS is the most sensitive assay for detection of endocrine disruption and this assay is preferred over the Two-Generation Reproductive Toxicity Study (OECD TG 416) (OECD, 2018).

The non-guideline study of Yang et al. (2010) examines *in vivo* effects of BADGE (unknown purity) in male rats with Gastric lavage with a single dose of 0, 500, 750, 1000 and 2000 mg/kg bw/day. An increased number of immature and maturing sperm in the testis was observed in the 750, 1000 and 2000 mg/kg bw/d groups. No significant differences in sperm head count, sperm motility, and sperm abnormality were observed. The authors conclude that a single oral exposure of BADGE 750 mg/kg bw/day can affect adult male testis development. They have included several endocrine sensitive endpoints, but the power of the study is low (with only five males in each group).

The eMSCA evaluated that the results of this non-guideline study (Yang et al., 2010) of testis toxicity to be not suitable for a reliable conclusion on the results and difficult to interpret due to the methodology used.

In conclusion, the registered substance BADGE (CAS RN 1675-54-3) does not adversely affect fertility in rats at the tested dose levels in two standard OECD guideline studies on reproductive toxicity performed according to older versions of the guidelines.

7.9.7.2 Developmental toxicity

The results of experimental studies investigating developmental toxicity are summarised in the following table:

Table 17: Overview of experimental studies on developmental toxicity

Method	Results	Remarks	Reference
Developmental/ teratogenic toxicity study **rat** (CrL:COBS CD (SD) BR) 25 dams in each dose group oral: gavage 0, 60, 180, 540 mg/kg/day (nominal conc.) Vehicle: 0.5% carboxymethylcellulose / 0.1% v/v Tween 80 solution Exposure: GD 6-15 (daily) **OECD Guideline 414** (Prenatal Developmental Toxicity Study) (1981)	NOAEL on maternal toxicity was 180 mg/kg bw/day (slightly retarded mean bodyweight gain) NOAEL on fetotoxicity: > 540 mg/kg bw/day (nominal) Pre-implantation loss was slightly higher in each of the treated groups compared with the controls. Differences were not strictly dosage-related or statistically significant (P>0.05). (*Pre-implantation loss = No. of corpora lutea –minus no. of implantations)/ no. of corpora lutea x 100*) The overall incidence of malformed foetuses was low, 0, 2, 3 and 1 in Groups 1 to 4 respectively. In addition, there were no adverse effects on embryo and foetal development as assessed by overall incidence and types of malformations, visceral and skeletal abnormalities at any dose level.	1 (reliable without restriction) key study Test material (EC name): 4,4'-Isopropylidenediphenol, oligomeric reaction products with 1-chloro-2,3-epoxypropane, DGEBA (CAS RN 25068-38-6) TK 10490, content of BADGE, CAS RN 1675-54-3) = 79.7%.	Unpublished study, 1988a
Prenatal Developmental Toxicity Study **OECD Guideline 414** **rabbit** (New Zealand White) oral: gavage N=18 Does pr dose group 0, 20, 60, 180 mg/kg bw/day (nominal conc.) Vehicle: 0.5% carboxymethylcellulose / 0.1% v/v Tween 80 solution in water Exposure: GD7-19 (daily)	The study showed evidence of maternal toxicity at 180 mg/kg/day in terms of anorexia and an initial weight loss. Ovulation rate (mean corpora lutea count, was similar in all groups) Pre-implantation loss slightly higher at 180 mg/kg/day Post implantation losses slightly higher in all treated groups. However, differences were not dose-related. Mean litter sizes slightly lower in all treated groups compared to the control group. None of these intergroup differences from the controls attained statistical significance. The incidence of malformed foetuses (e.g. One foetus with a sutural bone) was 6, 14, 8 and 2 in groups 1 to 4 respectively (from 5, 9, 5 and 2 litters respectively). The unusually high incidence at 20 mg/kg bw/day was considered fortuitous, by the research centre (CRO)and therefore not treatment-related, as the incidence at the higher dosages of 60 and 180 mg/kg/day were comparable with the controls.	1 (reliable without restriction) key study experimental result Test material (EC name): 4,4'-Isopropylidene diphenol, oligomeric reaction products with 1-chloro-2,3-epoxy-propane: DGEBA (CAS RN 25068-38-6) TK 10490, content of BADGE, CAS RN 1675-54-3 : 79.7%	Unpublished study report, 1988b

Method	Results	Remarks	Reference
One-generation study oral gavage rat (CrL:CD(SD) BR VAF/Plus) male/female N=25 dams pr. group 0, 20, 30, 180, 540 mg/kg/day (nominal conc.), analysed test concentrations: 13.1-17.4, 50.6-55.0, 154.8-166. and 464.4-499.0 mg/kg/day, (thus values were 8- 35% below that intended) Vehicle: 0.5% carboxymethylcellulose and 0.1% (v/v) Tween 80 OECD Guideline 415 (One-Generation Reproduction Toxicity Study) (1981). Exposure: Males were dosed 10 weeks prior to mating, through mating to termination (PND 21). Females were treated daily for two weeks prior to mating until termination following weaning of F1 offspring (PND 21). During the pre-weaning period all offspring were examined to determine the age at several developmental stages were attained e.g. Air righting reflex and Startle reflex. On Day 22 post partum, pups were sacrificed and examined externally and internally for abnormalities. Any tissues showing macroscopic abnormality were preserved.	Highest dose, (540 mg kg bw/ day): post-dosing salivation in all animals (F0), slightly reduced food consumption in females, (first week of treatment only), reduced body weights in males. 180 mg kg bw/day:post-dosing salivation and a slight decrease in food consumption in females (F0) during the first week of treatment. Pups: assessment of of pre-weaning development showed slight reduction in mean pup weights on days 12 and 21 post-partum at 540 mg/kg bw/d (however attributed to the higher litter size). Also air righting reflex was slightly delayed in both 180 and 540 mg/kg group which could be related to the reduced growth rate. No adverse findings on the developing foetuses were reported at any dose. Surface righting and air righting tests involve sensory and motor co-ordination systems.	This study is identical with the one gen-study described above under fertility. 1 (reliable without restriction) supporting study experimental result BADGE CAS RN 1675-54-3: 79.7% The OECD TG 415 from 1981 did not include endocrine sensitive parameters.	Unpublished study, 1989e

Method	Results	Remarks	Reference
Two-generation study rat (Sprague-Dawley) male/female Oral: gavage OECD Guideline 416 (Two-Generation Reproduction Toxicity Study, (before the update in 2001) 0, 50, 180, 540 or 750 mg/kg/day (nominal conc.) 30 male and 30 females per. group Immediately prior to breeding, a decision to terminate the 180 mg/kg/ day dose group was made. This decision was based on the fact that only three treatment groups are required by EPA guidelines, and the animals clearlv tolerated the 750 mg/kg/ day dose level without significant toxicity. Vehicle: aqueous solution of 0.5% Methocel A4M (Trademark, Dow Chemical) with 0.1% Tween 80 Exposure: 238 days total (once daily)	Body weights for adult males were decreased by approximately 8 to 11 % at dose levels of 540 and 750 mg kg body weight-1 day- 1 in both the F1 and F2 generations. In females, body weights were also affected in both generations, but only at the highest dose level (750 mg kg bw/day). Secondary changes in absolute and/or relative liver and kidney weights were also observed in these dose groups. There were no treatment-related effects on body weights among males given 50 mg kg bw/day or among females given 50 or 540 mg kg bw/day in either generation. There were no statistically significant treatment-related effects on reproductive performance and no treatment-related histopathological changes in any dose group. Evaluation of the various parameters of neonatal growth and survival over the three sets of litters produced by the two generations of adults revealed no indication of any significant adverse effect of DGEBPA at any dose level.	This study is identical with the two gen-study described above under fertility. 1 (reliable without restriction) key study OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) (1996) Test material BADGE (CAS RN 1675-54-3), of 99.65%	Unpublished study report, 1996 and 1997.

Method	Results	Remarks	Reference
***In vivo* effects on developmental and endocrine end-points in rats** Non-guideline study. Mated female rats, divided into four groups (n=12 per, group). Dosing solutions BADGE (purity unknown) in corn oil at the 0; 375; 1500 and 3000 mg/kg bw/day Mated females were dosed once daily (oral gavage) GD 6 - 20 and PND 0 - 21. All live animals were kept to PND 63. Pregnant female dams were observed for general symptoms and body weight. Male pups were observed for the general symptoms; body weight, developmental parameters (e.g. Anogenital distance, pina detachment, incisor eruption, nipple retention, eye opening, testis descent), organ pathologic changes and for hormone levels of plasma. Female rats only assessed for Sex ratio of live fetuses.	<u>Parental generation</u>: High lethality in 3000 (100%) and 1500 (70%) mg/kg bw/day dose groups (only one viable litter), but no toxicity at 750 or 375 mg/kg bw/dmg/kg bw/day. <u>Offspring:</u> 375 mg/kg bw/day: significantly lower body weights in male pups at PND 42, 56, and 63 ($p<0.05$) Evaluation of body characteristics including separation of auricle, eruption of incisor, separation of eyelid, nipple retention, descent of testis, and separation of the prepuce in the BADGE treated group showed no difference in comparisons with the control group. AGD (Anogenital distance) tended to be longer in males (375 mg/kg bw/day) however not significant. Testosterone in the treated pups (375) does not increase as much as in controls however the small number of animals probably contributed to high standard errors and the finding is not significantly different and may not be treatment related.	Reliability: weak Flawed study design: small numbers of animals in dose groups; dose selection, leading to high mortality in 2 doses) and statistical analyses (should be litter-based, but was pup-based). Thus the data from this study are not particularly robust for evaluation of BADGE *in vivo* developmental toxicity effects.	Hyoung et al 2007

Method	Results	Remarks	Reference
***In vivo* effects of BADGE on development of male rats (such as AGD and epididymis)** BADGE was administered orally to pregnant 8-week-old (SPF) Sprague-Dawley female rats from gestational day 6 to lactation once daily at doses of 0 (control), 50, 200, or 400mg kg bw/ day (5 dams per group) Five male pups in control and three treatment groups were culled and sacrificed by ether on postnatal days (PND) 21, 42, or 56. Body and epididymis changes in weight and developmental characteristics of each male pup were recorded. AGD was recorded on PND 4, 7, 14	The adjusted anogenital distance (AGD) on PND 7 in the 50 mg kg bw/day group was significantly shorter than in control animals; However, on PND 14 in the 200mg kg/day group and on PND 4 and 14 in the 400 mg kg/day group, AGD was significantly longer. Male rats treated with 50mg kg/day of BADGE exerted no effect on epididymis.	Reliability: weak Small number of animals (n=5), Kruskal–Wallis test (not taken litter into account in statistics) No Purity of BADGE provided The data from this study showed no clear monotonic dose-response *in vivo*	Kwon et al., 2010
Prenatal Developmental Toxicity Study Rabbit (New Zealand White) Exposure by **dermal** application to 0, 100, 300 and 500 mg/kg bw/day on GD 6 to 18 OECD TG 414	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicity and developmental toxicity = 500 mg kg body weight/day)*	Medium relevance due to dermal exposure (No info. on absorption across rabbit skin) and no ED parameters included in the studies	Unpublished report from 1986, cited in EU, 2002.
Prenatal Developmental Toxicity Study Rabbit (New Zealand White) pregnant Exposure by **dermal** application to 0, 30, 100 and 300 mg kg body weight/day on gestation days 6 to 18. OECD TG 414	No significant effects (relative to the controls) on embryo and foetal development at any test dose *(NOEL for foetotoxicity and developmental toxicity = 300 mg kg body weight/day)* Significant effects (relative to the controls) on pregnancy rate and foetal sex ratio at 30 mg kg body weight/day (but not in higher doses).	Medium relevance due to dermal exposure (No info. on absorption across rabbit skin) and no ED parameters included in the studies	Breslin *et al* (1988)

Summary and discussion of studies investigating effects on developmental toxicity

Rat and rabbit developmental toxicity studies have been conducted via the oral route, and two rabbit studies have been conducted via the dermal route. Information on developmental toxicity from the one- and two- generation studies in rats are also included in table 17 above and reported below.

The test guideline compliant oral and dermal prenatal developmental toxicity studies (OECD TG 414) on TK 10490[4] did not show cause for concern regarding foetal deaths, visceral or skeletal malformations or adverse effects on embryonal and foetal development.

Rats dosed orally on days 6 to 15 of gestation (OECD Guideline 414) had evidence of maternal toxicity at 540 mg/kg/day with slightly retarded mean body weight gain. A physiological change (increase in post-dosing salivation) which is not necessarily indicative of a toxic response per se was recorded at the high (540 mg/kg bw/d) and intermediate dosages (180 and 60 mg/kg bw/d). There was no evidence that treatment of the dam had any adverse effect on embryofoetal development or morphology at any of the dosages investigated (unpublished report (1988a). In QSARs predictions of reduced foetal weight were reported for BADGE, BADGE-mono-diol and BADGE-diol for fetal weight decrease in rodents (wt dec rodent (AI1)) in a Leadscope model.

Oral administration of TK 10490 to rabbits during days 7 to 19 of gestation produced evidence of maternal toxicity at 180 mg/kg/day in terms of anorexia and an initial weight loss. Treatment at 60 and 20 mg/kg/day was not associated with any maternal toxicity. There was no evidence that treatment of the dam with TK 10490 adversely affected mean litter parameters. Embryofoetal development and morphology was unaffected at all the dosages investigated (Unpublished report (1987)).

Based on the results from the two-generation reproductive toxicity study (OECD TG 416), the no-observed-effect level (NOEL) for adult males was considered 50 mg/kg/day based on body weight and body weight changes, and the NOEL for adult females was 540 mg/kg/day based on body weight changes at the higher dose group (750). The NOEL for reproductive effects was 750 mg/kg/day in this TG 416 study. Unfortunately, investigation of sperm parameters were not part of the TG 416 at the time of the test performance. The version of the TG 416 used in this study does not include e.g. onset of puberty, oestrus cycle and sperm parameters (e.g. sperm morphology and motility of P and F1 generation males). The OECD TG 416 was updated in 2001, including endocrine disruption sensitive end-point to include a more comprehensive range of endpoints particularly sensitive to EDs.

Significant effects on pregnancy rate and foetal sex ratios in New Zealand White rabbits exposed dermally to BADGE were found in a teratogenicity study at a dose of 30 mg/kg bodyweight/day, though effects were not evident at higher doses (100 and 300 mg/kg bw/d) (Breslin et al 1988). However, no effects on litter size or sex ratios were found in another study in rabbits (Breslin et al., 1986) nor was it found in rats at similar exposure doses (Smith et al. 1988a and 1988b).

The studies on dermal exposure in rabbits are difficult to interpret, as information on absorption across rabbit skin is unavailable. Toxicokinetic information from mice shows that absorption following dermal exposure to BADGE is low, with most of the dose remaining at the application site and/or its covering. The percutaneous absorption of BADGE in mice was slow. Moreover, these studies were not designed to detect endocrine active substances.

There are a number of published non-guideline studies (see table 16 and 17 above) examining *in vivo* effects of BADGE on reproductive and developmental toxicity (Hyoung et al., 2007; Kwon et al., 2010; and Yang et al., 2010) with a variety of study designs. The studies include several endocrine sensitive endpoints and indicate increased number of immature and maturing sperm in the testis. However, the power in the studies is low (in one study the number of animals included is 5) and the statistical analyses are incorrect (pup based instead of litter based). The studies have many deficiencies and unusual practices in relation to study designs and data are not robust enough for the evaluation of potential *in vivo* effects of BADGE. In addition, there are reports of contradictory results,

[4] DGEBA (CAS RN 25068-38-6) TK 10490, purity (n=0, CAS RN 1675-54-3) = 79.7%

which make them difficult to evaluate. The eMSCA evaluates that these studies cannot be used in the evaluation of reproductive toxicity or endocrine disruption.

Overall, there was no clear evidence of developmental toxicity in rats and rabbits following oral administration or in rabbits following dermal administration of BADGE. Slightly reduced body weight were seen in both the one- and two generation studies in the offspring of high dose animals and could be a developmental effect. The OECD states in its guidance document 43 from 2008 that a change in offspring body weight or weight gain is a sensitive indicator of developmental toxicity, in part because it is a continuous variable. In some cases, weight differences in offspring may be the only indicator of developmental toxicity in a generation study (OECD, 2008). However, the effects seen were not considered sufficiently severe to conclude on a developmental effect of BADGE.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

The registration dossier from 2015 included DNELs that were not derived following the recommendations of ECHA's *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).* The use of smaller AF was not justified by specific substance information in line with Annex I, 1.4.1 of the REACH regulation on the derivation of DNELs.

Having in consideration the severity of possible effects of the registered substance, a correct and justified DNEL setting is important. Consequently, the ECHA decision of 17 May 2017 included a request that the registrant should provide a "revision of Section 5.11 of 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 include a substance specific justification for using other AFs".

The decision further provided specification that the specification should include: a specification of how the following points 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.."

In their updated registration, the registrants have provided revised DNELs set in accordance with the ECHA guidance recommendations with respect to the use of assessment factors, leading to a reduction of the DNELs for inhalation and dermal long terme exposure of workers and the consumers.

The request in the substance evaluation decision of May 2017 is thus fulfilled, and no further information on this end-point is therefore necessary.

7.10. Assessment of endocrine disrupting (ED) properties

The eMSCA has evaluated the initial concern on endocrine disruption in man and in the environment related to the registered substance. The eMSCA reviewed the information

from the *in vivo* information (repeated dose toxicity studies, reproductive toxicity studies) included in the registration dossier. Moreover, *in vitro* and *ex vivo* data not presently included in the registration dossier and *in silico* information on BADGE and its major decomposition products were added.

Cytotoxicity stability/degradation

In an *in vitro* study by Russo et al. 2018, they tested the cytotoxicity of BADGE in four different cell lines: 3T3-L1, MCF-7, C6 and Hela and compared it with the cytotoxicity of BPA. BADGE was found to cause cytotoxicity in all four cell lines, and it was more cytotoxic than BPA in all tested cell lines (statistical significance not calculated) (Russo et al. 2018).

BADGE can be easily hydrolysed in contact with aqueous and acidic matrices, leading to the formation of BADGE·H20 and BADGE·2H20, the latter being the predominant in aqueous matrix food (Hammarling et al. 2000).
In a study conducted by Marqueño et al. 2019 they among other analyses tested the stability and availability of BADGE in cell culture medium as well as evaluated the cytotoxicity and ability of BADGE and two of its derivatives BADGE·H20 and BADGE·2HCl to disrupt CYP19 (aromatase) activity in the human placental choriocarcinoma cell line JEG-3.
BADGE and BADGE·H20 caused cytotoxicity in JEG-3 cells with an EC_{50} of 38-43 µM and 81-100 µM, respectively.
Inhibition of aromatase was also seen in the JEG-3, but the effect was mostly attributed to cytotoxicity, particularly in the case of BADGE.

In a study by Marqueño et al. 2019 the results indicate that BADGE is very unstable in cell culture medium. Thus potential effects seen *in vitro* in different cell-based assays are likely not be the effect of the parent compound BADGE, but a combination of the effects of its various breakdown products. In addition, the EC_{50} values based on nominal concentrations underestimate the toxicity of these compounds. Consequently, taking into account the experimental concentrations of the compounds instead of the nominal ones, BADGE and its derivatives show a much higher cytotoxicity and a greater ability to inhibit CYP19 activity (BADGE·H20) in JEG-3 than BPA (Marqueño et al. 2019).

Punt et al. 2019 investigated the breakdown of BADGE and its derivatives *in vitro*. They find extensive breakdown of BADGE, BADGE·H20 and BADGE·HCl following exposure to Caco-2 cells, resulting in low recoveries. In Punt et al. (2019) it is described that; BADGE first converts to BADGE·H20, then to BADGE·2H20. BADGE·H20 converts to BADGE·2H20 and BADGE·HCl is converted to BADGE·H20. This was similar in HepaRG cells.

TOXCAST data[5]**:**
When searching by CAS RN, BADGE (CAS RN 1675-54-3) in the available ToxCast database, BADGE have been tested in 60 ToxCast *in vitro* assays, and has been found active in 20 of the assays.
The assays were BADGE was found to be active (have effect) covers endpoints related to ER and AR activity, thyroid effects and aromatase inhibition. However, generally the effects observed in these assays was first seen at concentrations above the reported cytotoxicity limit, thus no conclusion regarding the effects and mechanisms of action can be drawn from the ToxCats data due to cytotoxicity.

Overall, the most pronounced effect seen *in vitro* for BADGE and several of its breakdown products is cytotoxicity. This makes it difficult it draw conclusions from potential antagonistic (anti-estrogenic and/or anti-androgenic) effects observed *in vitro*, as these effects could potentially be due to cytotoxicity and not a real antagonistic effect. Nevertheless, any antagonistic effect in these assays cannot be dismissed.

[5] The US EPA's contribution to Tox21 is ToxCast, is a battery of in vitro endocrine disruption assays used to develop activity signatures of chemicals. It is used for prioritisation for testing under Endocrine Disrupter Screening Programme (EDSP) (Reif et al. 2010).

7.10.1. Endocrine disruption human health - Estrogenicity

(Q)SAR predictions

Positive predictions were obtained for ER agonism *in vitro* in a DTU model based on US EPA data (CERRAP project with training set data based on integration of results from 18 ER related assays, see Mansouri et al. 2016) for BADGE-bis-diol. BADGE is included in the training set as a negative and the remaining substances were predicted positive but with probabilities outside the defined applicability domain (AD) (positive probability above 0.5 but below 0.7).

Experimental studies estrogenic endpoints (*in vitro* and *ex vivo*).

In the following *in vitro* assays investigation possible estrogenic effects are summarised, some in the table 18 and some below the table.

Table 18: Endocrine disruption; estrogenic *in vitro and ex vivo*

Method	Results	Remarks	Reference
Estrogenic effects were assessed using the E-Screen/MCF-7 bioassay, measuring cell proliferation as well as synthesis and secretion of cell type-specific proteins. A competitive binding assay based on cytosol from immature female rat uteri was used to study the relative binding affinity of BAGDE for ER.	In MCF7 cells BADGE behaved as a partial estrogen agonist, as it only increased cell proliferation below 60% of that found for E2 (the positive control), and it had no effect on induction of the cell type specific proteins: progesterone receptor (PgR) and pS2. In the competitive binding assay, BADGE showed no affinity for binding to the uterine ER.	Purity of BADGE was at least 97%. No cytotoxicity stated in the paper.	Perez et al. 1998
A hydrolysis (BAGDE-4OH) and chlorhydroxy (BADGE-2CL) derivative of BADGE were tested for estrogenic activity, using a T47D breast cancer cell proliferation assay. In addition, the derivatives were also tested in a commercially available human ERα binding assay.	Both derivatives showed **estrogenic activities**, and for both of them the measured activity were stronger than that of BPA measured in the same assay. The proliferation of BADGE-4OH and BADGE-2CL was inhibited by the addition of the ER antagonist ICI 182,780, confirming that the proliferation was mediated by ER. However, neither of the compounds was found to bind to the human ERα, and it was therefore suggested that the estrogenic activity of BADGE-4OH and BADGE-2CL is not caused by direct interaction with ER.	The purity of the BADGE-4OH and BADGE-2Cl standards is up to 97.0%. No cytotox stated in the paper.	Nakazawa et al. 2002
A sample of commercial BADGE was fractioned by HPLC and eight impurities were identified. The estrogenicity (agonism and antagonism) of fractions containing these impurities was measured using a yeast two-hybrid assay	Estrogenic antagonistic activity was found for two fractions, while no agonist activity was in any of the fractions. Binding affinity to hERα was found in three fractions. Altogether, the results were found to give rise to concern about impurities originating at the manufacturing process of BADGE, and it was concluded that a comprehensive	The purity of BADGE was 88% in one of the samples and unknown in all other samples. No	Terasaki et al. 2006

incorporating the human ERα (hERα). In addition binding to ER was assessed using a competitive binding assay for hERα.	assessment of the estrogenic properties of commercial BADGE, and their implications for human health, would require examination of all its components.	cytotox stated in the paper.	
The estrogenic and anti-estrogenic activity of BADGE was tested in an *ex vivo* study using the CARP-HEP/*vgt* assay. The CARP-HEP/*vgt* assay is based on the measure of ER-mediated vitellogenin production in hepatocytes from male carp (*Cyprinus carpio*).	BADGE was in this *ex vivo study* found to have ER-antagonistic effects in fish (*Cyprinus carpio*), as it was a potent antagonist of the ER-mediated vitellogenin production.	Purity of BADGE >99% Cytotoxicity is taken into account in the evaluation.	Letcher et al. 2005
The XenoScreen XL YES/YAS assay based on yeast cells (Saccharomyces cerevisiae) stably transformed with hERα (YES) was used to investigate estrogenic and anti-estrogenic activity.	BADGE demonstrated **weak anti-estrogenic activity**, with an 84% inhibition at 100 µM. For the hydrolysed product BADGE·2H2O estrogenic or anti-estrogenic activity was found.	Purity of BADGE ≥95.9% Cytotoxicity is taken into account in the paper.	Fic et al. 2014

In a report from 1998 (TemaNord, 1998) the effects of BADGE and its hydrolysis product [bisphenol A bis(2,3-dihydoxypropyl ether] was evaluated in a recombinant yeast screen (YES). The test showed no indication of estrogenic effect and no responses were recorded at concentrations ranging from 0.5 – 500 µM.

Szczepanska et al. 2018 and van Leeuwen et al. 2019 have tested the estrogenic and androgenic effect of BADGE and several of its derivatives using yeast bioassays (YES and YAS assay). No estrogenic nor androgenic activity could be found for BADGE or any of the tested derivatives.

In contrast to Szczepańska et al. 2018, Leeuwen et al. 2019 found weak anti-estrogenic activity (IC50 >50 µM) and weak anti-androgenic effect (IC50 >20 µM) of BADGE.
Similar to Szczepanska et al. 2018, Leeuwen et al. 2019 also found anti-estrogenic effect of BADGE·HCl, and BADGE·HCl·H20 (IC50 of 20 and 100 µM, respectively) as well as anti-androgenic activity (IC50 of 8 and >40 µM, respectively) (Leeuwen et al. 2019).

Ex vivo

Letcher et al. (2005) tested BADGE in two separate sets of 6-fold replicates. The use of fish hepatocytes to investigate estrogenic antagonism is well described in more than 30 peer reviewed studies and the paper publishing the method (Smeets et al. 1999) used by Letcher et al. (2005) has been cited more than 90 times and is reproducible (Web of Science 2015). Although the maximum solvent (DMSO) concentration in the test medium used by Lechter and co-workers (0.2%) was above the OECD recommended maximum solvent concentration in *in vivo* tests (OECD, 2000), no effect on cell viability was observed by determining changes in the mitochondrial succinate dehydrogenase-mediated metabolism of the substrate 3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT). Also BADGE did not affect MMT. The authors in Letcher et al. (2005) suggest that the estrogenic antagonistic effect of BADGE was caused by a direct interaction with the estrogen receptor. Since the hepatocytes have some metabolising capacity, the effect of BADGE in these cells may be induced by metabolites of BADGE, e.g. the BADGE-bis-diol, which in structure has close similarities with that of bisphenol A.

In conclusion, BADGE is observed to induce strong anti-estrogenicity in carp hepatocytes in Letcher et al, 2005. This may be induced by direct interaction with the estrogen receptor in these cells.
In mammalian cells, binding to hERα seems to be relevant for only some fractions of BADGE, and estrogenicity in mammalian cell proliferation assays, seem to be independent of binding to the ER in immature rat uteri and the hERα for BADGE and the BADGE-bis-diol respectively.

Some *in vitro* studies of BADGE showed weak anti-estrogenic activity, whereas other showed no estrogenic activity, maybe due to the cytotoxic potential of BADGE.

QSAR models showed some positive predictions for human ER binding *in vitro* for several BADGE compounds.

Several differences were observed between some of the (Q)SAR predictions on estrogenicity /training set and results from other studies *in vitro*/*ex vivo as mentioned above*. This may be due to the use of methods and cell types from several animal species, and dissimilar binding affinities to ER receptors (e.g. mammalian and fish ER receptors and their activation processes). In relation to the *in vitro* results it could also be due to occurrence and concentrations of constituents/impurities in different tested BADGE batches. Moreover the cytotoxicity seen in some of the *in vitro* assays could also lead to some of these discrepancies.

Experimental studies on estrogenic endpoints in vivo

Please see study summaries in section 7.9.4 (repeated dose toxicity) section 7.9.7 (Toxicity to reproduction), including summaries of the OECD TG 408 (90-day study), OECD TG 414 (Prenatal Dev. Tox study), OECD TG 415 (One generation reproductive toxicity study) and OECD TG 416 (Two generation) studies as well as the non-guideline studies performed with BADGE.

In the performed 90 day oral repeated dose toxicity study (doses 0; 50; 250 or 1000) (OECD TG 408) in rats, significant decreases in body weight and feed consumption were seen (see table 13). Some organ weights (absolute and relative) were affected: in males significant decreases in absolute adrenal gland, heart, and spleen weights, and increases in relative brain and testes weights were seen at 250 mg/kg bw/day. Based on alterations in body and organ weights and serum cholesterol in rats given 50 mg/kg/day, a no-observed-effect level (NOEL) was not determined. The increased cholesterol was interpreted by the registrant to be non-adverse because rats are relatively resistant to the induction of hyperlipidema and atherosclerosis even when serum cholesterol levels are elevated. The eMSCA finds that the cholesterol increases raise some concern, as humans may be more sensitive to increased serum cholesterol levels than rats. Unfortunately cholesterol have not been assessed in the 2 generation study or any other studies in the registration dossier.
Endocrine organs and hormone sensitive tissues were affected at 1000 mg/kg bw/day, with degeneration of the seminiferous tubules in the testes and atrophy of the endometrium of the uterus. These effects could be induced by a general toxic effect, since the animals also suffered from decreased body weight and food consumption, and effects were also observed in several organs and tissues not being specific to the endocrine system.
The NOEL for responses in endocrine organs or hormone sensitive tissues (degeneration of the seminiferous tubules in the testes and atrophy of the endometrium of the uterus at 1000mg/kg bw/day) in both male and female rats was for this study 250 mg kg bw/day.

The OECD test guideline (TG) 408 (Repeated Dose 90-Day Oral Toxicity Study in Rodents) has been updated in 2018 with several endocrine endpoints after this study was performed. In the update the measurement of T4, T3, TSH and thyroid gland weight is required. In addition, serum total cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) should also be determined as levels of these parameters are directly

controlled by thyroid hormone action and contribute (with other thyroid endpoints) to evidence of thyroid effects. Optional endpoints include other hormone measurements, as well as assessments of sperm parameters. Negative existing *in vivo* effects data from studies performed prior to the update should be interpreted with caution as an a priori conclusion that the effects do not present a concern for endocrine disruption. This should be held up against the fact that the results may rather reflect that relevant endocrine endpoints (such as thyroid hormone levels) were not investigated, or that the tests used do not have sufficient power to detect weak effects (OECD GD 150, 2018). Even though a number of longer term repeated dose and also reproductive toxicity studies (part 7.9.7) are available in rodents, none of them clarify the concern regarding possible endocrine disrupting effects of BADGE.

The reported 2-generation study (OECD TG 416) on BADGE is conducted in 1996. No adverse effects were observed on reproductive performance. OECD TG 416 was revised in 2001 to include a more comprehensive range of endpoints, so the available 2-generation study on BADGE is using an old version of TG 416. Consequently, sensitive parameters for endocrine disruption such as assessment of sexual maturation (VO and PPS) effects on sperm quality and estrus cyclicity in offspring (update TG 416, 2001) were not included. Also, there were no recording of developmental milestones including some optional behavioural parameters and histopathology of sex organs, brain and identified target organs (OECD GD 43, 2008). It is also noted that according to OECD ED Conceptual Framework in GD 150 (OECD 2018), as well as in REACH annexes the OECD TG 443 (extended one generation reproduction toxicity) is the preferred test over TG 416 for identification of endocrine disruptors because it includes some ED related parameters not included in the current OECD 416 (2001).
An OECD TG 415 study from 1989 was performed on DGEBA (79.7% BADGE) with doses of 0, 20, 60, 180, or 540 mg/kg bw/d. No treatment-related macroscopic changes, differences in mean organ weights or histological changes to the reproductive tract (only assessed in top dose) in either sex of the F0 generation were observed. The one-generation assay (OECD TG 415) has now (since Dec. 2019) been deleted as it has been made redundant following the introduction of the Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) (OECD, 2018), which includes several ED relevant parameters.

The OECD two-generation and one-generation reproduction toxicity studies are apical assays designed to provide general information concerning the effects of a chemical on the male and female reproductive systems including gonadal function, the oestrus cycle, mating, conception, gestation, parturition, lactation, weaning and growth and development of the offspring. The studies are not specifically designed to detect EDs but they have many endpoints relevant for the assessment of possible endocrine disruption and provide data on adverse effects related to reproduction and development. The one-generation study (OECD TG 415), adopted in 1983, only includes one cycle of mating and is much less prescriptive in both the performance of the study and the endpoints to be assessed. It has now been deleted (as of dec. 2019 as mentioned above), but was earlier placed at Level 4 when the Conceptual Framework was revised in 2011 (OECD 2012a). In contrast, the two-generation study (OECD TG 416) includes two cycles of mating and the original OECD TG 416 was revised in 2001 to include a more comprehensive range of endpoints. These endpoints include sexual maturation (VO and PPS) which are particularly sensitive to EDs. One-generation studies and two-generation studies conducted prior to the adoption of the revised OECD TG 416, like in this case with BADGE, are therefore unlikely to provide as much data as studies conducted to the revised OECD TG 416, particularly with respect to endocrine disruption. They do however provide a great deal of useful data, particularly on adverse effects on reproduction (OECD, 2012a). Among the current OECD, test guidelines for mammalian reproductive toxicity, exposure during all vulnerable periods of development is performed in the Extended
One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) and the Two-Generation Reproductive Toxicity Study design (OECD TG 416). The EOGRTS is the most sensitive assay for detection of endocrine disruption and this assay is preferred over the Two-Generation Reproductive Toxicity Study (OECD, 2018).

In OECD TG 414 studies in rats and rabbits from 1988 on DGEBA with 79.7% BADGE, only none dose-related effects on prenatal development that do not reach statistical significance up to doses of 180 mg/kg bw/day were observed. However, no ED sensitive endpoints were investigated, as the 414 guideline at that time did not include such end-points. TG 414 was in 2018 updated to also include anogenital distance in the fetuses as well as thyroid hormones in the dams (and possibility for other hormones).

The published non-guideline studies (reported and described below and under 7.9.7) examine *in vivo* effects of BADGE on reproductive and developmental toxicity, including several endocrine sensitive endpoints (Hyoung et al., 2007; Kwon et al., 2010; and Yang et al., 2010). The study designs vary greatly. The power of the studies is low and the statistical analyses are incorrect (pup based instead of litter based). The studies have many deficiencies and unusual practices in relation to study designs, and some reports of contradictory results.

In Hyong et al (2007), in which BADGE of unknown purity is administered to pregnant rats on GD 6-20 and PND 0-21 at 0, 375, 1500, 3000 mg/kg bw/d, no effects of ED concern are observed (e.g. in male pups AGD, nipple retention, testis descent, hormone levels in plasma, organ pathologic changes or preputial separation). However, there was high lethality in the 3000 (100%) and 1500 (70%) mg/kg bw/d dose groups, and the body weight in male pups was significantly decreased in the 375 mg/kg/d group. Further, there was a small number of animals in the study. Moreover, non-litter based statistics were used in this study which can result in an inflated sample size. It is critical that littermates are not treated as independent observations in the statistical analysis as litter should be the statistical unit (OECD GD 43, 2008).

In Yang et al., (2010), which tested BADGE of unknown purity in 5 male rats per group with gastric lavage with a single dose of 0, 500, 750, 1000, 2000 mg/kg bw, an increased number of immature and maturing sperm in the testis was observed in the 750, 1000 and 2000 mg/kg bw groups. The authors conclude that a single oral exposure of BADGE 750 mg/kg and above can affect adult male testis development. No significant differences in sperm head count, sperm motility, and sperm abnormality were observed. The eMSCA evaluated that the results of this non-guideline study of testis toxicity to be not suitable for a reliable conclusion on the results and difficult to interprete due to the methodology used.

In Kwon et al., (2010), which tested BADGE of unknown purity in 5 pregnant rats per dose group dosed GD6-lactation with 0,50,200,400 mg/kg/d, a shorter AGD was observed at PND7 in the 50 mg/kg/d group, and a longer AGD was observed at PND14 in the 400 mg/kg/d group in male offspring. However, a small number of animals per dose group (n=5), and the lack of monotonic dose-response make conclusions uncertain.

Even though a number of longer term repeated dose and reproductive toxicity studies are available in rodents, none of them clarifies the concern regarding possible estrogenic/anti-estrogenic effects of BADGE/DGEBA. The available *in vivo* studies in rodents are either conducted according to old OECD test guidelines, which do not include investigation of a number of important sensitive ED specific endpoints, or, for the non-guideline studies, with designs of the studies not suitable for a reliable conclusion on the results.

The effect of BADGE on the uterus and vagina of ovariectomized ICR mice was investigated in an *in vivo* Uterotrophic assay by Ogata and coworkers in 2001 and reported as a posted abstract by EU_WRC_report (2002)[6] . The mice received subcutaneous injections of BADGE at doses up to 1mg/kg bw for 3 consecutive days (0.1, 1, 10, 100 and 1000µg BADGE/kg). All the mice were then killed 24 hours after receiving the final injection. The uterus and vagina were removed, weighed and subjected to histopathological examination. No differences in organ weights or tissue histopathology were found between the negative

[6] This Poster abstract is reported in EU_WRC_report (2002). http://ec.europa.eu/environment/chemicals/endocrine/pdf/wrc_report.pdf

control (DMSO treated) animals and the BADGE treated mice. In contrast, positive control mice treated with 100 µg kg bw of 17β-oestradiol showed positive signs of estrogenic effects characterized by increased uterine weights and high grade stratification/cornification of the vaginal epithelium. Based on these data the authors of the study concluded, that BADGE does not exert estrogenic responses on the uterus and vagina in ovariectomized mice at relatively low doses.

Conclusion on concern regarding estrogenicity/anti-estrogenicity of BADGE

In conclusion, information from the available long-term mammalian *in vivo*, fish *ex vivo*, various *in vitro* and *in silico* data raises some concern for estrogenicity/anti-estrogenicity of the constituents and/or metabolites of BADGE.

QSAR models showed some positive predictions for human ER binding *in vitro* for several BADGE compounds and TG 408 showed some changes in some reproductive organ weights and an increase in cholesterol that could be ED mediated.

Many of the MoA studies *in vitro* do not show clear estrogenic effect. A number of *in vivo* studies in rats are available. However, since they either do not include the most sensitive ED relevant endpoints or are non-guideline studies for which the designs make the conclusions uncertain, estrogenicity/anti-estrogenicity in mammals cannot be excluded completely, and a residual concern subsists.

In addition, there is a concern for estrogenicity/anti-estrogenicity in fish based on a strong effect in one *ex vivo* study with BADGE in carp hepatocytes (Letcher et al. 2005), which is not clarified, since there are no available *in vivo* studies on potential estrogenicity/anti-estrogenicity in fish.

However, due to the high toxicity of BADGE in fish, there is most probably no currently recognised guideline that would be sufficiently sensitive to measure endocrine end-points *in vivo* at concentrations that would not cause toxicity.

7.10.2. Endocrine disruption - Anti-androgenicity

Anti-androgenicity of BADGE and BADGE diols and BADGE-N=1, BADGE-N=1-mono-diol and BADGE-N=1–bis-diol

(Q)SAR:
Positive predictions were obtained in a Leadscope DTU model for AR antagonism (*in vitro*) based on US EPA data (CoMPARA project with training set data based on integration of results from 11 AR related assays Mansouri et al. 2020) for BADGE and BADGE mono-diol.
Negative predictions were obtained in a Leadscope DTU CoMPARA model for AR agonism (*in vitro*) for BADGE, BADGE- mono-diol and BADGE-bis-diol, BADGE being included in the training set as a negative.
Positive predictions were obtained in a Leadscope DTU CoMPARA model for AR binding (*in vitro*) for BADGE mono and bis-diol, except for BADGE, which is included in the training set as a negative.

Experimental studies on (anti-)androgenic endpoints *in vitro*

In the following *in vitro* assays investigation possible anti-androgenic effects are summarised.

Table 19: Endocrine disruption; anti-androgenic *in vitro*

Method	Results	Remarks	Reference
The AR-EcoScreen, based on CHO-K1 cells stably transfected with human AR, was used to test for	BADGE was found to possess weak anti-androgenic activity with an IC_{50} above 100 µM. No reports of cytotoxicity.	Purity of BADGE >97%	Satoh et al 2004

androgenic and anti-androgenic effects.			
The XenoScreen XL YES/YAS assay based on yeast cells (Saccharomyces cerevisiae) stably transformed with hAR (YAS) was used to investigate androgenic and anti-androgenic activity.	BADGE demonstrated weak anti-androgenic activity with an 89 % inhibition at 100 µM. For the hydrolysed product BADGE·2H2O no androgenic or anti-androgenic activity was observed. Cytotoxicity is taken into account in the paper. (*Fic also mentioned in Table 18)*	Purity of BADGE ≥95.9%	Fic et al. 2014

In vitro

For BADGE, no reliable effect is obtained in an AR EcoScreen using Chinese Hamster Ovary K1 cells with human AR (Satoh 2004) (table 19) .
In a YAS assay, Fic, 2014 found anti-androgenic activity for BADGE, but no activity was observed for the BADGE-bis-diol (Fic, 2014) (table 18 and 19).
In contrast to Szczepanska et al. 2018, Leeuwen et al. 2019 found weak anti-androgenic effect (IC50 >20 µM) of BADGE.

Ex vivo

Desdoits-Lethimonier et al. (2017) wanted to address the question: "Are bisphenol A (BPA) and BPA analogues (BPA-A) safe for male human reproductive function?" They used adult human testis explants in culture that were exposed to BPA and its analogues bisphenol including bisphenol A diglycidyl ether (BADGE) at 10^{-9}, 10^{-9}, and 10^{-5}M for 24 or 48 h. BADGE leads to significantly decreased testosterone levels by 12.3% at 10^{-9}M and 28.8% at 10^{-7}M after 24 h and by 19.1% after 48 h of culture with 10^{-8}M. However, a relatively low number of testes samples were available for analysis (n = 3). The significant BADGE induced
suppression of testosterone found in this study occurred for exposure at lower concentrations (generally 10−6–10−8 M) than that observed for BPA. Whether the BADGE-induced anti-androgenic effect represents an intrinsic effect of these compounds is not known. The actual risk of exposure to BADGE is not clarifyied.

Experimental studies on (anti-)androgenic endpoints *in vivo*

Please see study summaries in section 7.9.4 (repeated dose toxicity) section 7.9.7 (Toxicity to reproduction), including summaries of the performed OECD TG 408, OECD TG 414, OECD TG 415 and OECD TG 416 as well as non-guideline studies.

It is important to bear in mind that older reproductive toxicity studies (e.g. TG 416) that lack these sensitive endpoints cannot exclude the possibility that chemicals not showing adverse effects on reproductive parameters included may still be endocrine disrupters.

The rodent *in vivo* database does not clarify whether the potential anti-androgenic activity is observed for BADGE *in vitro* could lead to adverse effects in rodents. The older OECD TG 408, 415 and 416 do not investigate the most sensitive endpoints in this respect (e.g. effects on AGD and nipple retention in male offspring exposed in utero), and the non-guideline studies are all flawed in their designs and do not show any clear dose responses (see description of studies under 7.10.1. estrogenicity/anti-estrogenicity).

Conclusion on concern regarding anti-androgenicity of BADGE

In conclusion, information from *in vitro* and *in silico* data raises a concern for anti-androgenic effects of the constituents and metabolites of BADGE.

A number of *in vivo* studies in rats are available, but since they either do not include the most sensitive ED relevant endpoints or are non-guideline studies with flaws in the designs, which make the conclusions uncertain, the data are not suited to evaluate a possible anti-androgenic effects of BADGE, and thus, there is a residual concern for anti-androgenicity in mammals.

In addition, there is a concern for anti-androgenicity in fish (see above and section 7.10.5 on endocrine disruption in the environmental), which is not clarified, since there are no available *in vivo* studies on potential anti-androgenicity in fish however, toxicity would probably preclude the possibility of obtaining such results.

None of the available rodent *in vivo* data resolves the concern on anti-androgenicity.

7.10.3. Endocrine disruption - Effects on the thyroid hormone system

Effects on the thyroid hormone system of BADGE and BADGE mono- and -bis-diol

(Q)SAR/in silico:
Positive predictions were obtained in a Leadscope DTU model for Thyroid Peroxidase (TPO) antagonism (*in vitro*) (Rosenberg et al. 2017) for all 11 substances.

***In vitro*:**
An *in vitro* study with BADGE (and metabolites) investigating the thyroid-disrupting mode of action of BADGE has been performed. The results are presented below.

Table 20. Overview of experimental studies on thyroid endpoints *in vitro*

Method	Results	Remarks	Reference
A human transthyretin (T4-TTR) competition binding assay was used to evaluate binding of BADGE and other related compounds to transthyretin (TTR).	Neither BADGE, BADGE·2H2O, nor BADGE·2HCl was found to be able to compete with T4 binding to TTR, when testing up to 5000 nM		Meerts *et al*., 2000

No competition with T4 of binding to TTR was observed for BADGE or the BADGE-bis-diol (Meerts et al., 2000), see table 20 above. No information is available on the registered substance, and limited *in vitro* information was identified on BADGE and its potential for interaction with the thyroid hormone system. The conclusion based on this study (*in vitro* T4-TTR binding) is that BADGE is not active with key thyroid system elements. However, this *in vitro* assay only examines one mechanism of action (TTR-binding) whereas other (not yet guideline) assays could have derived other thyroid mechanisms of action.

Experimental studies on thyroid endpoints *in vivo*

No histopathology was performed on the thyroid gland in the 2-generation study in rats (from 1998) conducted on the registered substance BADGE described under point 7.9, as this procedure was not part of the former OECD TG 416 (added in 2001).
The OECD TG 408 90-day study performed with BADGE described under point 7.5, reported all histopathologically examined thyroid glands to be within normal limits. Further thyroid

endpoints were included in the 2018 update of the TG 408, but the performed TG 408 study was made according to old version of the TG.
In the carcinogenicity studies (oral and dermal) no effects on thyreoidea was reported.
Further, no effect was observed on thyroid histopathology in the 2 generation study, TG 416, and no effect was observed on thyroid weight in the 90-day study, TG 408. However, serum thyroid hormone levels were not measured in any of the rodent studies.
Based on the current data from *in vivo* repeated dose toxicity studies in rats, BADGE appears not to target the thyroid system. However, both OECD TG 408 (before 2018) and TG 416 (version before 2001) do not examines effects on the thyroid system such as thyroid gland weights and histopathology and do not measure thyroid hormones. Serum hormone levels were included in the novel TG 443 – extended One Generation study and the updated TG 408 from 2018.
Moreover, no investigations of potential effects on thyroid hormone signalling were available from e.g. amphibians.

Effects on behaviour and brain development

Miyazaki et al. 2020 examined the effects of BADGE exposure to the dams on the behavioural, structural, and developmental abnormalities in the offspring (mice). They examined several behavioural tests (open field, elevated plus maze).
Female pregnant mice were in this study fed with a diet containing BADGE (0.15 or 1.5 mg/kg/day) during gestation and lactation periods. In an open field test, the time spent in the corner area was significantly increased in male mice of high-dose BADGE group at 5 weeks old. Moreover, they examined the effects of high-dose BADGE exposure on brain development by histological analysis using brain slices of neonatal mice at PD1. They observed effects of BADGE high-dose exposure during embryonic period on the cortical structure of neonatal mice. The authors conclude that these data suggest that maternal BADGE exposure can accelerate neuronal differentiation in fetuses and induce anxiety-like behaviour in juvenile mice.
These findings support BADGE related effects on the developing mouse brain.

Moreover, published data regarding BPA-related effects in rodents generally suggests heightened anxiogenic activity (Wolstenholme et al., 2011), a factor which contributed to the conclusion by the National Toxicology Program and the World Health Organization that there is some concern for BPA-related effects on brain and behaviour, particularly anxiety (Beronius et al., 2010; FAO/WHO, 2011).

However, the study by Miyazaki et al., 2020 only includes three litters in each dose group (very limited) and there are only three males in the high dose group where they report the behavioural effect. There only seems to be minor gender differences in the controls (if any) and it is unclear if they have studied it. It is not known if the findings are related to ED as suggested by the authors and the limitations in the study are too many to place too much emphasis on the findings in this study.

Conclusion on concern regarding effects on the thyroid hormone system of BADGE

Based on the currently available data, with the caution that sensitive thyroid ED endpoints were only scarly addressed, there are no indications of a potential to affect the thyroid hormone system.

7.10.4. Other ED MoAs (including effects on steroidogenesis)

Endocrine disruption; steroidogenesis

Letcher et al. (2005) assessed the effect of BADGE (purity of >99%) on aromatase (CYP19) activity in the human adrenocortical H295R carcinoma cell line, after exposing the cells to [1β-^{3}H(N)]-androst-4ene-3,17-dione and measuring its CYP19 catalysed conversion to astrone and $^{3}H_2O$. No effect of BADGE on CYP19 activity was found in this study. Other

effects of BADGE in the Letcher et al. (2005) study is also described under part 7.10.1 and in table 18.

PPARγ:

Table 21: PPAR activation

Method	Results	Remarks	Reference
Activation of PPARγ was assessed using ECV-ACO.Luc cells, a clone of ECV304 cells that have endogenous PPAR receptors and respond to known PPARγ agonists.	BADGE was found act as PPARγ agonist in µM concentrations in the ECV-ACO.Luc cells.		Bishop-Bailey et al., 2000
Differentiation of the 3T3-L1 and 3T3-F442A preadipocyte cell lines was used to study the effect of BADGE on PPARγ activity. Binding to PPARγ was assessed using a ligand binding assay for PPARγ, and finally a transcription reporter assay, based on NIH-3T3 cells transfected with plasmids encoding full-length PPARγ, RXRα, β-galactosidase and a DR-1 luciferase reporter was used to examine the effect of BADGE on the transcriptional activity of PPARγ.	BADGE was reported to act as a PPARγ antagonists in 3T3-L1 and 3T3-F442A preadipocyte cells. In the NIH-3T3 transcription reporter gene assay it is found that BAGDE does not activate the PPARγ/RXRα heterodimer, but instead seems to have PPARγ antagonistic effects, as BADGE was able to attenuate Rosiglitazone (a PPARγ agonist) induced transactivation to some degree. So, currently the mechanism by which BADGE should cause inhibition of PPARγ is not known.	The results from the 3T3-L1 and 3T3-F442A differentiation cannot be used to conclude anything about a PPARγ antagonistic effect of BADGE, as they actually do not see an inhibition of differentiation with BADGE.	Wright et al. 2000
Multipotent mesenchymal stromal stem cells (MSCs) and 3T3-L1 pre-adipocytes were used to study the effect of BADGE and others on adipogenesis. PPARγ activity was examined using a transfection assay based on COS7 cells transfected with human PPARγ and RXRα.	BADGE was found to elicite a dose-dependent conversion of human MSCs into adipocytes. When testing BADGE in in 3T3-L1 pre-adipocytes BADGE showed significant induction of adipogenesis at 10 nM. Thus BADGE was found to induce both MSCs and pre-adipocytes to undergo adipogenesis. Moreover, antagonizing PPARγ did not block the adipogenic effects of BADGE, and BADGE was neither found to activate nor antagonize PPARγ. The authors conclude that BADGE is likely to act through a pathway downstream of, or parallel to PPARγ.		Chamorro-García et al. 2012

Based on the available literature (see table 21 above) BAGDE appears to be a PPARγ antagonist in NIH-3T3 cells, and a relative potent agonist in the ECV304 cell line. Control

of the activation state of nuclear hormone receptors/transcription factors such as the PPARγ/RXR heterodimer is due to a large dynamic complex of proteins, and the differences between the reported effects of BADGE on PPARγ could be to cell type specific differences in the regulation of PPARγ activity. Finally, according to ToxCast data from 2013, BADGE does not bind to the human nuclear PPARγ (hPPARγ).

Bone related effects

The background for including bone measurements in the analysis of BADGE as an endocrine disruptor is that bones also produces hormones and is additionally a sensitive hormone target tissue. It is mentioned in several of the papers that BADGE is a PPARγ antagonist. PPARγ is involved in fatty acid metabolism, and genes of osteoclasts, apoptosis and cell signalling generally with growth factors. Despite this context, it is not directly useful in the evaluation of BADGE as a possible endocrine disruptor. In addition, the bone is not mentioned as a hormone target tissue in OECD GD 150 (OECD, 2018). The data below are therefore included to ensure a comprehensive assessment of possible influence of BADGE on endocrine relevant organs.

Four papers are dealing with bone related issues (Skeletal Health, osteoporosis, or bone quality) and BADGE. None of them has a focus on assessing the ED effect of BADGE. The four papers are briefly reported below:

The Wang et al (2019) study investigated the preventive effects of BADGE on steroid-induced
osteoporosis in mice. They find that BADGE treatment improves glucocorticoid-induced osteoporosis by inhibiting PPARγ in that animal model.

The Chin et al. review (2018) summarize the current evidence on the relationship between BPA (and derivatives) and bone health derived from cellular, animal and human studies. They conclude, that BPA and its derivatives (including BADGE) could influence bone health and a possible gender effect was observed in animal studies. However, due to the cellular and animal model used in the investigations, the skeletal effects of BPA and its derivatives are heterogeneous, whereby both positive and negative effects have been reported. A possible gender effect of BPA on bone has been revealed in animal studies (beneficial in males, deleterious in females).

The Yuan et al. study (2018) investigated the preventive effects of PPARγ inhibition on steroid-related osteonecrosis in a rabbit model. The authors report that, BADGE-treated rabbits exhibited reduced marrow adiposity associated with improved bone formation. They conclude that these observations demonstrated that pharmacological inhibition of PPARγ might represent an effective therapy for steroid-related osteonecrosis in the near future.

The Li et al. paper (2016) investigated the impact of BADGE on a relevant physiological model, the ovariectomized (OVX) rat model. They concludes that early BADGE treatment at a dose of 30 mg/kg weakens marrow adiposity in ovary-intact and OVX rats and stimulates bone formation in ovary-intact rats but does not significantly rescue bone quality in OVX rats. One solid point in the study is that they used a longitudinal design method to assess the sequential changes of BADGE on marrow fat content. One weakness is only a single standard dose of BADGE was used in this study.

Other endocrine mediated effects

Using isothermal titration calorimetry (ITC) Zhang et al. 2016 found BADGE·HCl·H20 to be a weak binder to thyroid hormone receptor THRβ1 (Zhang et al. 2016).
Finally, using the AMPHiTox test (an eco-tox test) Wolkowicz et al 2016 found BADGE to cause developmental toxicity in Rhinella arenarum, being more toxic to embryos than to larvae at all exposure times (Wolkowicz et al 2016).

Positive predictions were obtained in Leadscope DTU models for human pregnane X receptor (PXR) binding (*in vitro*) and for human PXR reporter gene (*in vitro*) for BADGE.

Positive predictions were obtained in a Leadscope DTU model for human constitutive androstane receptor (CAR) (*in vitro*) agonism at maximum 20 µM (i.e. highest potency model developed) for BADGE, BADGE mono-diol and BADGE diol.

Negative predictions were obtained in two Leadscope DTU models (final "Rational" and "Random" (Klimenko et al. 2019) for human arylhydrocarbon receptor (AhR) agonism (*in vitro*) for the predicted substances.

In conclusion, there is evidence from a number of studies that that BADGE is a PPARγ antagonist. PPARγ is involved in fatty acid metabolism and in bone development.

7.10.5. Endocrine disruption in the environment/Ecotoxicity

The results from the *in silico* and *in vitro* studies presented in the present substance evaluation in paragraph 7.10.1, 7.10.2, 7.10.3 and 7.10.4 above are relevant to wildlife as well as to human health and should therefore be taken into account in overall evaluation of the endocrine disruptive properties of BADGE.

In addition, information on mechanisms of action of BADGE as available from rodent *in vivo* studies is relevant to many other vertebrate species as well. This is due to the fact that hormone receptors and pathways are highly conserved across vertebrate species and cross-species extrapolations should be considered (OECD, 2018). Adverse effects seen in rodents would therefore also be relevant especially to mammalian wildlife species with low reproductive output including predators, and larger mammalian species (including endangered species), because any negative effect on development or reproduction has a high likelihood of leading to serious effects at the population level for such species. However, the available reproduction toxicity studies on BADGE were conducted according to guidelines not including endocrine toxicity sensitive endpoints.

To clarify the concern for anti-estrogenicity in fish, a Fish Sexual Development Test (TG 234) including the optional endpoint histopathology would be relevant, as it examines the sexual development of both male and female fish including vitellogenin concentrations and gonadal development as well as the sex ratio which is an adverse endpoint at the population level in fish. Taking this uncertainty into account, together with the *ex vivo* data on fish hepatocytes described above where BADGE (>99%) was a potent antagonist of vitellogenin production with an IC50 of 5.5 µM and virtually 100% inhibition of vitellogenin at 20 µM (Letcher et al 2005, see table 18 above), there is a concern that BADGE has an estrogenic antagonistic potential. However, as described the relatively high toxicity of BADGE in fish might likely be a problem and with the sensitivities of the endpoints currently included in FSDT it is therefore not likely to find any response. As stated in the report entitled "Information/testing strategy for identification of substances with endocrine disrupting properties" (Hass et al. 2013a)*:*

"Any alert should trigger further testing, taking into account exposure scenario and tonnage level. If clear alerts of estrogenic agonism, androgenic agonism or steroidogenesis inhibition appears from QSAR, chemical categorization, in vitro and/or toxicological in vivo studies, and for which aquatic exposure is possible, it is proposed to request the a FSDT (TG 234), where MoA (vitellogenin induction or reduction) may be related to adverse effect on phenotypic sex ratio. If the evidence of e.g. oestrogenic activity is weak, the Fish Short Time Reproduction Assay (TG 229) or the 21-day Fish Assay (TG 230) could alternatively be triggered in accordance with the OECD Fish Testing Strategy Guidance Document (OECD 2012, STA 171). These tests can be used as screening tests, which can inform about in vivo oestrogenic/androgenic MoA before it is decided whether to conduct the longer and more thorough and potentially confirmatory FSDT. In conclusion, it should be noted that currently the FSDT (TG 234) is the only validated OECD TG on non-mammalian vertebrate species which can inform about both endocrine activity and consequential adverse effects, i.e. if positive it can be used for a definitive identification of endocrine disrupters" (from Hass et al. 2013).

No long-term fish toxicity studies are available for BADGE or BADGE-related resins. The report thus recommends that a long-term fish test including vitellogenin as endpoint (TG 229, TG 230, TG 234 or TG 240) should be performed to clarify the concern for endocrine disruption. Of these, the TG 234 (FSDT) contains the most sensitive E/A/S related endpoints. However, it is noted that still T-endpoints would not be clarified, as there are currently no T-endpoints included in the above mentioned TGs.

Further, the relatively high toxicity of BADGE in fish might likely be a problem for the performance of *in vivo* tests. LC50(96h) has been measured to be 1.62 mg/L in a freshwater fish, meaning that the compound can only be tested in concentrations up to 0.16 mg/L in the FSDT. With the sensitivities of the endpoints currently included it is therefore not likely to find any endocrine responses at concentrations that do not cause toxicity. The EU_WRC_report on endocrine disrupters for the priority listing of actions (EU, 2002) describe the lack of fish testing as an area of uncertainty in relation to endocrine mediated effects: "No comparisons could be made for fish due to the absence of data on endocrine mediated responses in this taxonomic group, which represents an area of uncertainty."

The EU_WRC_report (EU, 2002) describe the lack of data on terrestrial exposure routes as an area of uncertainty: "*Given the potential for BADGE to bind to organic carbon the absence of data on potential terrestrial exposure routes represents an area of uncertainty with regard to the potential endocrine effects of the substance on wildlife*".

As described above the TG 234 (FSDT) contains the most sensitive E/A/S related endpoints and would be relevant. However, the relatively high toxicity of BADGE in fish might likely be a problem and with the sensitivities of the endpoints currently included in FSDT it is therefore not likely to find any response on endocrine end-points at concentrations not causing toxicity.

7.10.6. Endocrine disruption - Overall conclusion regarding concern for ED effects of BADGE

Based on the available *in vitro* data of BADGE (up to May 2020) the most pronounced effect of BADGE as well as its derivatives is cytotoxicity, an effect that is also supported by the ToxCast data.
BADGE seems to have some endocrine disrupting abilities, as especially the derivative BADGE bis-diol has anti-androgenic as well as anti-estrogenic potential in QSAR models. Based on the newest *in vitro* data BADGE does not seem to have androgenic nor estrogenic activity. BADGE looks to be very unstable in culture media, and is quickly hydrolyzed leading to the formation of hydrolysed derivatives e.g. BADGE·H20.
Differences were observed in the (Q)SAR predictions and training set experimental results and results from other studies *in vitro/ex vivo.* This may be due to the use of methods and cell types from several animal species, and dissimilar binding affinities to ER receptors (e.g. mammalian and fish ER receptors and their activation processes). In relation to the *in vitro* results it could also be due to occurrence and concentrations of impurities in different tested commercial DGEBA/BADGE.
Increased cholesterol levels seen in a sub-chronic study (TG 408) study may also be an indication of an endocrine effect of the bisphenol A moiety of BADGE. However, cholesterol was not assessed in other available *in vivo* studies with BADGE, and no conclusion on the significance of theses cholesterol levels can be drawn based on the available information.
Effects have also been reported on the activity of PPARγ, however these effects appears to be somewhat cell type specific. On the other hand, BADGE does not seem to affect key thyroid system elements.
However, there are currently several endpoints related to endocrine disrupting effects that have not been assessed both *in vivo* and *in vitro* (such as changes in hormone levels).

The available studies on BADGE do no show effects in some endocrine related endpoints/test, however, the non-guideline studies either do not investigate the most sensitive endpoints for endocrine MoAs, or are unreliable due to flawed and limited test

design. The *in vivo* guideline studies available have been performed at a time when ED sensitive endpoints were not included in the TG.

It is therefore currently not possible to exclude the initial concern that BADGE is an endocrine disruptor. Rather, some concern remains based on mammalian studies of repeated dose and reproductive toxicity *in vivo* that lack ED sensitive endpoints, from limited positive *ex vivo* data in fish and in human testis, from *in vitro* assays showing cytotoxicity in some doses and some *in silico* data showing estrogenic/anti-estrogenic and anti-androgenic effects of the constituents and/or metabolites of BADGE. On the other hand, a request for further testing in mammals would mean a high degree of redundancy with existing studies, and would be disproportionate having regard to principles of reduction of use of laboratory animals.

In addition, no studies on potential effects on estrogenicity/anti-estrogenicity or anti-androgenicity in ED relevant fish *in vivo* studies (e.g. OECD 229 or 234) are currently available and it is in relation to concerns for endocrine disruption in fish noted that there is indeed positive *ex vivo* data available indicating potential for (anti)estrogenic effects in fish. However, the high toxicity in fish most probably would preclude obtaining measurements of ED activity given the sensitivities of the currently applied endpoints in a possible follow-up *in vivo* test.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

7.12.1. Human health

Exposure of workers can occur amongst others at specialized industrial facilities or by widespread use by trained professionals. Exposure to consumers is expected e.g. from DIY renovation or craft projects. Substance release and exposure to the environment is also foreseen.

Due to the low concentrations of free unreacted BADGE in all end-use applications, concerns regarding serious risk from exposure to the substance were mainly raised for exposure to workers.

Workers exposure

The registered substance may pose a serious risk for human health and a concern was raised that workers were not properly informed to use the right type of PPE (gloves, goggles, masks and coveralls) to protect themselves against exposure. Hence, further information in the CSR on specifications of the advised personal PPE was requested in the ECHA substance evaluation decision of 17 May 2017.

As specified in the decision, the registrant was requested to provide further specification on PPE and the duration of use for all scenarios where the use of PPE was advised. In particular the type of material, thickness and breakthrough times of the gloves and the duration of use was requested. Furthermore, the registrant was asked to add information on sufficient protection of the body (coverall) or to justify why this is not advised in the relevant exposure scenarios.

Following the decision, the registrant committed to include more detailed specification on the use and type of PPE for workers in a future update of the dossier. The CSR has afterwards been updated accordingly.

Along with the newest update of the CSR in accordance with the decision, the estimated protection level of the advices gloves has been specified for the individual exposure scenarios. In the previous version of the CSR, the protection level from protectives gloves was set to be 90 % in all exposure scenarios. In the newest update of the CSR, the protection level of gloves is in some scenarios estimated to be as high as 99 %. Such values are usually not considered realistic and are not justified within the documentation of the used model (ECETOC TRA v3; the highest value for professional settings being 90% with basic employee training and 95% for industrial settings along with special employee training). The unusually high level of protection from protective gloves as estimated in the updated version of the CSR was justified by assuring intensive training for safe use of gloves and supervision of workers.

As some of the calculated RCR values in the substance CSR are very close to 1, the eMSCA is concerned about the applied estimated protection levels assumed to be achieved from wearing protective gloves. To achieve this unusually high level of protection from gloves, the eMCSA expects that information on how to achieve this high level of protection by using PPE is thoroughly passed on through the supply chain.

Consumers exposure

The eMSCA notes that the registrant recognizes consumer use and includes consumer exposure in the CSR.

The registrant stresses that all products for consumers are labelled with use instructions to ensure wearing of gloves and eye protection during use as well as advice on good general ventilation (by e.g. open window). All contributing scenarios thus consider minimal opportunity for contact with the product via skin, eyes and inhalation.

It is usually not considered realistic to assume that all consumers will use gloves - even when they are instructed so. It is the registrants responsibility to ensure minimal opportunity for contact with the substance in this regard, and it is important that the information on PPE for consumer use is very explicit and thoroughly delivered through the supply chain.

7.12.2. Environment

Not evaluated.

7.12.3. Combined exposure assessment

Not evaluated.

7.13. Risk characterisation

Not evaluated.

7.14. References

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