Substance Name: 1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione) (ß-TGIC)

EC Number: 423-400-0

CAS Number: 59653-74-6

SUPPORT DOCUMENT FOR IDENTIFICATION OF

1,3,5-TRIS-[(2S AND 2R)-2,3-EPOXYPROPYL]-1,3,5-TRIAZINE-2,4,6-(1H, 3H, 5H)-TRIONE)

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS CMR[1] PROPERTIES

[1] CMR means carcinogenic, mutagenic or toxic for reproduction

CONTENTS

TABLES AND FIGURES

Tables

Substance Name(s): 1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione)

EC Number(s): 423-400-0

CAS number(s): 59653-74-6 (ß-TGIC)

The substance is identified as a substance meeting the criteria of Article 57 (b) of Regulation (EC) 1907/2006 (REACH) owing to its classification as mutagenic category 1B, which corresponds to classification as mutagen category 2[2].

Summary of how the substance meets the criteria as category 1A/B carcinogen, category 1A/B mutagen and category 1A/B reproductive toxicant.

1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione (ß-TGIC) (EC number: 423-400-0, CAS number: 59653-74-6, index number for the Annex VI entry: 616-091-00-0) is listed in Annex VI, part 3, Table 3.1 of Regulation (EC) No. 1272/2008 (list of harmonised classification and labelling of hazardous substances) as mutagen category 1B (H340; May cause genetic defects). This corresponds to a classification in Annex VI, part 3, Table 3.2 (the list of harmonised and classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 as mutagen category 2 (R46; May cause heritable genetic damage).

Therefore, this classification of the substance in Regulation (EC) No 1272/2008 means that it meets the criteria for classification in the hazard class germ cell mutagenicity category 1B in accordance with Article 57 (b) of REACH.

Registration dossiers submitted for the substance? No

Remark: This supporting document is very different from the original Annex XV dossier for ß-TGIC due to the additional information from the industry received as comments. Based on the new data ß-TGIC is imported to EU in a preparation and is used exclusively under strictly controlled conditions as solder mask ink. All other workers exposure scenarios mentioned in the previous version of this document are not valid anymore.

[2] Classification in accordance with Regulation (EC) No 1272/2008 Annex VI, part 3, Table 3.1 List of harmonised classification and labelling of hazardous substances.

Justification

1 Identity of the substance and physical and chemical properties

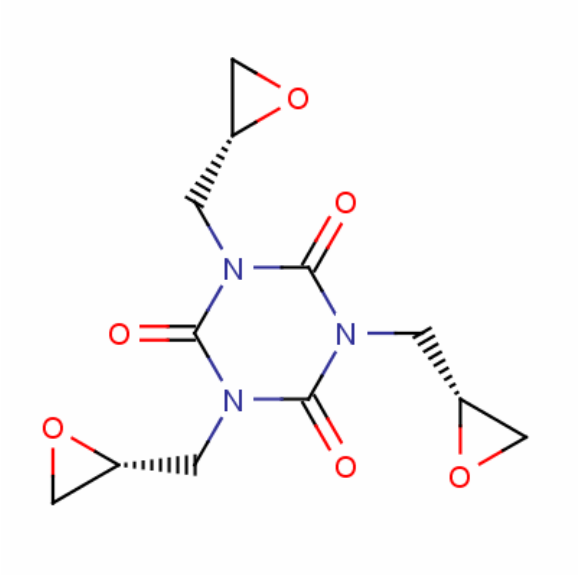
1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	423-400-0
EC name:	1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione
CAS number (in the EC inventory):	59653-74-6 (ß-TGIC)
CAS name:	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-tris[(2R)-2-oxiranylmethyl]-, rel-
IUPAC name:	reaction mass of 1,3,5-tris[(2R)-oxiran-2-ylmethyl]-1,3,5-triazinane-2,4,6-trione and 1,3,5-tris[(2S)-oxiran-2-ylmethyl]1,3,5-triazinane-2,4,6-trione
Index number in Annex VI of the CLP Regulation	616-091-00-0
Molecular formula:	C12H15N3O6
Molecular weight range:	297.3 g/mol
Synonyms:	triglycidyl isocyanurate TGIC; 1,3,5-triglycidyl isocyanurate; 1,3,5-triglycidyl-s-triazinetrione; 1,3,5-tris(2,3-epoxypropyl)-s-triazine-2,4,6(1H,3H,5H)- trione; tris(2,3-epoxypropyl)isocyanurate ß—Triglycidyl isocyanates NSC 296964 TEPIC-H

(Registration Dossier 2010), (Nordic Council of Ministers 2001)

Structural formula:



1.2 Composition of the substance

Name: 1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione

Description: epoxidized triazine

β-TGIC is a monoconstituent isomeric substance with low level of α-TGIC present as an impurity. (Industry comments on Annex XV dossier)

Degree of purity: ***Confidential information***

1.3 Physicochemical properties

Table 2: Overview of physicochemical properties

Property	Value	Reference
Physical state at 20°C and 101.3 kPa	solid, powder	Data from notification under the Directive 67/548/EEC
Melting/freezing point	156 °C	Data from notification under the Directive 67/548/EEC
Boiling point	Decomposing at 262 °C at 1013 hPa	Data from notification under the Directive 67/548/EEC
Vapour pressure	< 2 x 10^{-6} Pa at 25 °C	Data from notification under the Directive 67/548/EEC
Water solubility	1010 mg/l at 20 °C; pH ca. 6.2	Data from notification under the Directive 67/548/EEC
Partition coefficient n-octanol/water (log value)	0.457 at 20 °C	Data from notification under the Directive 67/548/EEC
Dissociation constant	pKa is not applicable for ß-TGIC	ß-TGIC has no functional groups to dissociate, it remains in water as parent molecule or is hydrolyzed, depending on pH.

2 Harmonised classification and labelling

1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione is covered by Index number 616-091-00-0 in Annex VI, part 3 of Regulation (EC) No 1272/2008 as follows:

Table 3: Classification according to part 3 of Annex VI, Table 3.1 ((list of harmonised classification and labelling of hazardous substances) of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	Classification		Labelling		
		Hazard Class and Category Code(s)	Hazard statement code(s)	Pictogram, Signal Word Code(s)	Hazard statement code(s)	Suppl. Hazard statement code(s)
616-091-00-0	1,3,5-tris-[(2*S* and 2*R*)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1*H*, 3*H*, 5*H*)-trione	Muta. 1B Acute Tox. 3 Acute Tox. 4 STOT RE 2 Eye Dam. 1 Skin Sens. 1	H340 H331 H302 H373 H318 H317	GHS06 GHS08 GHS05 Dgr	H340 H331 H302 H373 H318 H317	-

*** Hazard class + category**	**Hazard statement + Text of the hazard statement**
Muta. 1B:	H340 (May cause genetic defects)
Acute Tox. 3	H331 (Toxic if inhaled.)
Acute Tox. 3	H302 (Toxic if swallowed)
STOT RE 2	H373 (May cause damage to organs through prolonged or repeated exposure)
Eye Dam. 1	H318 (Causes serious eye damage.)
Skin Sens. 1	H317 (May cause an allergic skin reaction)

**** Hazard statement code:**
H317: May cause an allergic skin reaction. (by the oral and inhalation route)
H340: May cause genetic defects
H318: Causes serious eye damage
H301: Toxic if swallowed.
H331: Toxic if inhaled
H373: May cause damage to peripheral lymph system

Table 4: Classification according to part 3 of Annex VI, Table 3.2 (list of harmonised classification and labelling of hazardous substances from Annex I of Council Directive 67/548/EEC) of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	Classification	Labelling
616-091-00-0	1,3,5-tris-[(2*S* and 2*R*)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1*H*,3*H*,5*H*)-trione	Muta. Cat. 2; R46 T; R23 Xn; R22-48/22 Xi; R41 R43	T R: 46, 22, 23, 41, 43, 48/22 S: 53, 45

*__Classification:__

T; R23 Toxic; Toxic by inhalation.
Xn; R22-48/22 Harmful; Harmful: danger of serious damage to health by prolonged exposure if swallowed.
Xi; R41 Irritant; Risk of serious damage to eyes.
R43 May cause sensitisation by skin contact.
Muta. Cat. 2; R46 May cause heritable genetic damage.

Labelling:
T - toxic
R41 - risk of serious damage to eyes
R23 - toxic by inhalation
R43 - may cause sensitisation by skin contact
R48/22 - harmful: danger of serious damage to health by prolonged exposure if swallowed
R46 - may cause heritable genetic damage
S45 - in case of accident or if you feel unwell, seek medical advice immediately (show the lable where possible)
S53 - avoid exposure - obtain special instructions before use

(Source: ESIS 2012)

3 Environmental fate properties

Not relevant for the identification of the substance as SVHC in accordance with Article 57(b).

4 Human health hazard assessment

See section 2 on harmonised classification and labelling.

For details on the relevant Human Health endpoints see Annex 1.

5 Environmental hazard assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57(b).

6 Conclusions on the SVHC Properties

6.1 PBT, vPvB assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57(b).

6.2 CMR assessment

1,3,5-tris-[(2S and 2R)-2,3-epoxypropyl]-1,3,5-triazine-2,4,6-(1H, 3H, 5H)-trione (ß-TGIC) (EC number: 423-400-0, CAS number: 59653-74-6, index number for the Annex VI entry: 616-091-00-0) is listed in Annex VI, part 3, Table 3.1 of Regulation (EC) No. 1272/2008 (list of harmonized classification and labeling of hazardous substances) as mutagen category 1B (H340; May cause genetic defects). This corresponds to a classification in Annex VI, part 3, Table 3.2 (the list of harmonized and classification and labeling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 as mutagen category 2 (R46; May cause heritable genetic damage).

Therefore, this classification of the substance in Regulation (EC) No 1272/2008 means that it meets the criteria for classification in the hazard class germ cell mutagenicity category 1B in accordance with Article 57 (b) of REACH.

6.3 Substances of equivalent level of concern assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57(b).

7 References

Nordic Council of Ministers, 2001. 128 - Triglycidyl isocyanurate. The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. Number 2001:18 Nordic Council of Ministers. http://www.inchem.org/documents/kemi/kemi/ah2001_18.pdf, accessed 10th December 2010.

Registration Dossier 2010, Registration dossier of TGIC (mixture of isomers)

ESIS, 2012, ESIS, Accessed on internet 19-01-2012, http://esis.jrc.ec.europa.eu/index.php?PGM=cla

ANNEX I Relevant Human Health Endpoints

1. Toxicokinetics (absorption, metabolism, distribution and elimination)

No information on β-TGIC toxicokinetics is available. Data for α-TGIC is used as an indication.

Several biochemical and clinical studies indicate that α -TGIC is rapidly absorbed by the oral and inhalation route (mutagenicity studies), but that dermal absorption is slow and less efficient. Nevertheless, dermal absorption takes place as indicated by the moderate skin sensitization potential in experimental animals and by the numerous case reports on Human skin sensitization.
Once absorbed, TGIC is rapidly metabolized by epoxide hydrolases, in most of the organs and tissues of vertebrates, most efficiently in humans.
No bioaccumulation has been observed in humans during clinical trials, and recovery was fast after the end of treatment. In experimental animals treated for 90 days or 2 years no bioaccumulation was observed either.
TGIC is distributed via blood in the entire body causing effects in blood cells, liver, lymph system as well as in peripheral tissues. Metabolites are mainly the hydroxylates (either di-, tetra-or hexa-hydroxylated TGIC). No parent compound has been found in urine of humans.
In conclusion, TGIC is absorbed rapidly, distributed and metabolized in short time(hydrolysis half-life in humans < 2 minutes) and excreted within 24 hours. No bioaccumulation has been observed in experimental animals or in humans.

Non-guideline target-oriented studies have been conducted to investigate the influence of epoxide hydrolase and other enzymes on the hydrolysis and detoxification of TGIC, on the DNA-binding potential of TGIC, and in clinical trials to elucidate the potential anti-tumour activity of TGIC in humans. Epoxide hydrolase is the key enzyme to hydrolyse TGIC in many organs of the animal and human body. It forms the respective triols which are glucuronidated and excreted. Degradation / hydrolysis of TGIC also occurs in the stomach due to low pH of 1-3. The alkylation potential is rapidly eliminated by acid treatment of TGIC, thus, the mutagenic potential is dependent on the intact TGIC-molecule (hydrolysis products are inactive). Human clinical studies (Phase-1) have shown that the anti-tumour activity found in mice was lacking in Humans. This is due to the very short half-life of TGIC in the humans (t/2 < 2minutes).

The following toxico-kinetic picture of TGIC can be drawn, based on studies summarized above and repeated dose toxicity studies:

- TGIC is rapidly absorbed from the lung, and the gastro-intestinal tract, but slowly and to a small extent from skin.
- In the stomach it is hydrolyzed by acid and in the organism by epoxide hydrolases.
- The serum half-life of the substance is <2 minutes; is metabolized to a large extent to a triol cyanurate, which is rapidly excreted.
- After oral exposure, the maximum blood levels are reached after 2-4 hours with a rapid decline afterwards.
- Due to the short serum half-life, no organ defects are found after acute exposure (oral, dermal, inhalation).
- Only after repeated exposure, hematological effects and effects on the lymph nodes, spleen and thymus are found. The same is true for effects in spermatogonial cells which appear only after repeated exposure. Based on its half-life in the organisms and based on the logPow (-0.8) no bioaccumulation is expected.

(Registration Dossier 2010)

Mechanism of toxicity: No information is available concerning the mechanism of toxicity of TGIC. Considering that TGIC contains three reactive epoxide groups it is plausible that it reacts with macromolecules causing different adverse effects, e.g. inducing mutations by binding directly to DNA and sensitization by binding to proteins. Dose dependent increases in TGIC-

DNA adduct formation were reported in a non peer reviewed study. (Nordic Council of Ministers 2001)

2. Mutagenicity

This is an overview of data for the mixture TGIC (α and ß TGIC; technical grade TGIC contains 90% α -and 10% ß-isomer). As there is no data for pure ß-TGIC, information on TGIC mixture is given. Both mixture TGIC and ß-TGIC are classified as Mutagen Category 1B (mutagen category 2).

2.1 Non-human information

2.1.1 In vitro data

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

Table 5: Overview of experimental in vitro genotoxicity studies

Method	Results	Remarks	Reference
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) S. typhimurium TA 1538 (met. act.: with and without) Doses: 1.22 – 10000 microgram/plate OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Test results: positive for S. typhimurium TA 1535; met. act.: with and without; cytotoxicity: yes positive for S. typhimurium TA 98; met. act.: with and without; cytotoxicity: yes positive for S. typhimurium TA 100; met. act.: with and without; cytotoxicity: yes ambiguous for S. typhimurium TA 1538; met. act.: with and without; cytotoxicity: yes	2 (reliable with restrictions) key study experimental result	A.J.W. Hoorn (1987)
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: with and without) Doses: Seven TGIC-concentrations were tested (15.63 1000 g/ml) in the first and second (0.47 - 30 g/ml) test. TGIC without S9 (0.175, 0.35, 0.7, 1.4, and 2.8 g/ml) TGIC with S9 (0.375, 0.75, 1.5, 3.0, and 6.0 g/ml) method according to Clive, D. et al Validation and characterization of the L5178Y/TK+/- mouse lymphoma mutagen	Evaluation of results: positive (with and without metabolic activation) Test results: positive for mouse lymphoma L5178Y cells(strain/cell type: 2) TGIC was dissolved in DMSO, and diluted into the culture medium. 3) In a preliminary toxicity test the concentration of TGIC causing a 85% reduction of the viability of cells was determined in suspension growth after 4-hour treatment and 72 hour susp); met. act.: with and	2 (reliable with restrictions) key study experimental result	P. Beilstein & D. Müller. (1983)

Method	Results	Remarks	Reference
assay system. Mutation Res. 59, 61-108 (1979)	without; cytotoxicity: yes		
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Doses: 312.5 - 5000 micrograms/plate JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals	Test results: positive for S. typhimurium TA 1535; met. act.: with and without; cytotoxicity: no, but tested up to precipitating concentrations positive for S. typhimurium TA 100; met. act.: with and without; cytotoxicity: yes positive for E. coli WP2 uvr A; met. act.: with and without; cytotoxicity: yes negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100; met. act.: with and without; cytotoxicity: yes positive for S. typhimurium TA 98; met. act.: with and without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result	PC Jenkinson (1988a)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) E. coli WP2 uvr A (met. act.: with and without) Doses: 312.5 - 5000 micrograms/plate JAPAN: Guidelines for Screening Mutagenicity Testing Of Chemicals	Test results: negative for S. typhimurium TA 1537; met. act.: with and without; cytotoxicity: yes positive for S. typhimurium TA 1535; met. act.: with and without; cytotoxicity: yes positive for S. typhimurium TA 98; met. act.: with and without; cytotoxicity: yes positive for S. typhimurium TA 100; met. act.: with and without; cytotoxicity: yes positive for E. coli WP2 uvr A; met. act.: with and without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result	PC Jenkinson (1988b)
in vitro mammalian	Evaluation of results:	2 (reliable with	F. Strasser & P.

Method	Results	Remarks	Reference
chromosome aberration test (chromosome aberration) lymphocytes: Human (met. act.: with and without) Doses: 0.0625, 0.125, 0.25, 0.5, 1.0 g/ml without S9-activation 0.625, 1.25, 2.5, 5.0, 10.0 g/ml with S9-activation Method according to Obe, G. , Beek, B., Vaidya, VG. (1975). The Human Leucocyte test system. III. Premature chromosone condensation from chemically and X-ray induced micronuclei. Mutation Research 27, 89-101	positive Test results: positive (upper two dose levels) for lymphocytes: Human; met. act.: with and without; cytotoxicity: yes	restrictions) key study experimental result	Arni (1985)
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair) primary culture, other: Human fibroblasts (CRL 1521, passage no. 11, 18) (met. act.: without) Doses: Toxicity test: 7.81 1800 g/ml DNA-damage test: 250, 100, 30, 9, and 2.7 g/ml. performed according to the published method of San, R.H.C. and Stich, H.F. DNA repair synthesis of cultured human cells as rapid bioassay for chemical carcinogens. Int. J. Cancer 16, 284-291 (1975)	Evaluation of results: negative Test results: negative for mammalian cell line, other: Human fibroblasts (CRL 1521, passage no. 11, 18) (strain/cell type: Human fibroblasts (CRL 1521, passage no. 11, 18)); met. act.: without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result	Th. Hertner and E.
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair) primary culture, other: rat hepatocytes (met. act.: without) Doses: 28.1 1750 g/ml for cytotoxicity testing 0.2, 1, 2.5, 5, 10 and 20 g/ml for the main test	Evaluation of results: positive Test results: positive for hepatocytes: rat(strain/cell type: primary rat hepatocytes); met. act.: without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result	Th. Hertner and E. Puri (1988)

Method	Results	Remarks	Reference
OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro)			
mammalian cell gene mutation assay (Transformation test) BALB/3T3 mouse embryo fibroblasts, clone A31-1-1 (T. Kakanuga NCI NIH, Bethesda, USA). (met. act.: without) Doses: 3.75 ng/ml 1000 g/ml (toxicity rest) 8.75 140 ng/ml (transformation test). Transformation assay in BALB/3T3 mouse embryo Fibroblasts. Transformation requires gene mutations to abolish contact inhibition between fibroblast cells.	Evaluation of results: negative Test results: negative for BALB/3T3 mouse embryo fibroblasts(strain/cell type: BALB/3T3 mouse embryo fibroblasts); met. act.: without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result	P. Beilstein & D. Müller (1983)

(Registration Dossier 2010)

2.1.2 In vivo data

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

Table 6: Overview of experimental in vivo genotoxicity studies

Method	Results	Remarks	Reference
mammalian germ cell cytogenetic assay (chromosome aberration) mouse (strain B6D2F1) male oral: gavage 0, 28.75, 57.5, and 115 mg/kg (analytical conc.) OECD Guideline 483 (Mammalian Spermatogonial Chromosome Aberration Test)	Evaluation of results: positive Test results: Genotoxicity: positive (increase in major chromosomal aberrations) (male); toxicity: yes (change of cytotoxic ratio)	1 (reliable without restriction) key study experimental result	R. Marchall (1991)
micronucleus assay (chromosome aberration) hamster, Chinese (, raised in Ciba-Geigy premises as outbred strain) male/female oral: gavage 0, 140, 280, and 560	Evaluation of results: positive Test results: Genotoxicity: positive (male/female); toxicity: no effects	2 (reliable with restrictions) key study experimental result	G. Hool & P. Arni (1983a)

Method	Results	Remarks	Reference
mg/kg (nominal conc.) The methods applied in this study are referenced as follows: Boller, K. & Schmid, W. Humangenetik 11, 35-54 (1970); Matter, B. & Schmid, W. , Mutation Research 12, 417-425 (1971); Müller, D. et al., Verh. Dtsch. Ges. Path. 56, 381-384 (1972).			
chromosome aberration assay (chromosome aberration) mouse (strain B6D2F1 (hybrid of C57B1/6 x DBA/2)) male oral: gavage 0 (control), 28.75 mg/kg (low dose), 57.5 mg/kg (intermediate dose), and 115 mg/kg (high dose), (analytical conc.) OECD Guideline 483 (Mammalian Spermatogonial Chromosome Aberration Test)	Evaluation of results: positive Test results: Genotoxicity: positive (male); toxicity: no effects (no clinical signs recorded, no bw changes)	1 (reliable without restriction) key study experimental result	R. Marshall. (1991)
chromosome aberration assay (chromosome aberration) mouse (male Crl:CD-1(ICR)BR mice) male inhalation: dust 0, 1.79, 10.3, and 49.6 mg/m3 air (analytical conc.) EPA OTS 798.5380 (In Vivo Mammalian Cytogenetic Tests: Spermatogonial Chromosomal Aberrations)	Evaluation of results: positive Test results: Genotoxicity: positive (at 10.3, and 49.6 mg/m3 air) (male); toxicity: yes (loss of weight during exposure period)	2 (reliable with restrictions) weight of evidence experimental result	J.J. Vergnes & E.R. Morabit. (1992a)
sister chromatid exchange assay (chromosome aberration) hamster, Chinese male/female oral: gavage 0. 140, 280, and 560 mg/kg (nominal conc.) no guideline cited ,but performed according to Allen, J.W. et al, Cell	Evaluation of results: positive Test results: Genotoxicity: positive (male/female); toxicity: no effects	2 (reliable with restrictions) supporting study experimental result	G. Hool & P. Arni (1983b)

Method	Results	Remarks	Reference
Genetics 18, 231-237, 1977, and Marquardt, H. & U. Bayer, Mutation Research 56, 169-176, 1978, Chinese hamster bone marrow cells in-vivo were evaluated with respect to Sister chromatid exchange (SCE).			
mammalian germ cell cytogenetic assay (chromosome aberration) mouse (CD-1) male inhalation: dust 1.79, 10.3, and 49.6 mg/m3 air (mean gravimetric measurements) EPA OPPTS 870.5380 (In Vivo Mammalian Cytogenetic Tests: Spermatogonial Chromosomal Aberrations)	Evaluation of results: ambiguous Test results: Genotoxicity: negative (male); toxicity: yes (decreased mitotic index, insufficient analysable metaphases)	2 (reliable with restrictions) supporting study experimental result	J.J. Vergnes & E.R. Morabit. (1992b)
micronucleus assay (Nucleus anomaly Test) hamster, Chinese male/female oral: gavage 0, 140, 290 and 560 mg/kg bw (nominal conc.) no guideline cited, but the method used is Matter, B. and Schmid, W. Mutation Research 12, 417-425 (1971). Study on interphase nuclei in bone-marrow cells of Chinese hamster after oral exposure (gavage) of a single dose.	Evaluation of results: positive Test results: Genotoxicity: positive (single Jolly bodies increased) (male/female); toxicity: no effects	2 (reliable with restrictions) supporting study experimental result	G. Hool & P. Arni (1983d)

(Registration Dossier 2010)

2.2 Human data

There are no Human mutagenicity data available.

2.3 Summary and discussion of mutagenicity

Discussion

TGIC mixture of isomers has been shown to cause gene mutations in-vitro in bacterial systems as well as in mammalian cell cultures systems.
It also caused chromosomal aberrations, micronuclei, and sister chromatid exchanges in mammalian cell systems.

In-vivo, TGIC mixture of isomers caused in a variety of rodent assays chromosomal aberrations, in both somatic as well as in germinal tissues.
The reason for the lack of gene mutations in-vivo is not known (Mouse Gene Mutation Spot test, which is not summarized in the table due to the reliability 3 score), but it could have many reasons: Either the systems used were not sensitive enough for gene mutations, or the major mutagenic activity of TGIC mixture of isomers is to cause chromosomal aberrations, e. g. DNA breaks and not base modifications or base substitutions.
However, the observed effects are significant and make TGIC mixture of isomers a category 2 mutagen (according to 67/548/EEC classification) or mutagen 1B (according to CLP classification).

The following information is taken into account for any hazard / risk assessment:
TGIC mixture of isomers is genotoxic in-vitro and in-vivo.
It causes chromosomal effects in male germinal tissues such as testis and seminiferous tubules.
Primary and secondary spermatocytes are affected.
This conclusion is also used for β-TGIC.

Justification for classification
Based on the in-vitro mutagenic effects and based on the in-vivo clastogenic effects in somatic as well as in germ cells the classification and labelling as category 2 mutagen and R46 or mutagen 1B and H340 (according to CLP classification) is justified.

(Registration Dossier 2010)

Remark: This is an overview of data for mixture TGIC (α and ß TGIC; technical grade TGIC contains 90% α -and 10% ß-isomer). As there is no data for pure ß-TGIC, information on TGIC mixture is given. Both mixture TGIC and ß-TGIC are classified as Mutagen Category 1B (mutagen category 2).