Substance Name: 1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione (TGIC)

EC Number: 219-514-3

CAS Number: 2451-62-9 (TGIC, a combination of two isomers)

SUPPORT DOCUMENT FOR IDENTIFICATION OF

1,3,5-TRIS(OXIRAN-2-YLMETHYL)-1,3,5-TRIAZINANE-2,4,6-TRIONE

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

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

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Substance Name(s): 1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione

EC Number(s): 219-514-3

CAS number(s): 2451-62-9

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(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione (EC number: 219-514-3, CAS number: 2451-62-9) is covered by index number 615-021-00-6 of Regulation (EC) No. 1272/2008 in Annex VI, part 3, Table 3.1 (the list of harmonised classification and labelling of hazardous substances) as mutagenic, Muta. 1B (H340; May cause genetic defects). The corresponding classification in Annex VI, part 3, Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 is mutagenic, Muta. Cat. 2, (R46; May cause genetic defects").

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

Therefore, this classification of the substance in Regulation (EC) No 1272/2008 shows that it meets the criteria for classification as mutagenic in accordance with Article 57 (b) of REACH.

Registration dossiers submitted for the substance? Yes

Remark: TGIC mixture of isomers (CAS nr 2451-62-9) is also submitted for Substance Evaluation as scheduled for 2013 by the Polish Competent Authority. The Industry has recognized that the available worker exposure data is outdated, and acknowledged the necessity to obtain occupational exposure monitoring data relating to the applications of TGIC in the European Union. Therefore the Industry will collect monitoring data and report this to support the planned Substance Evaluation for 2013.

[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

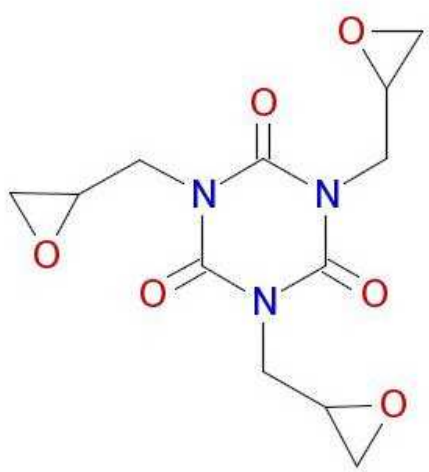
1 Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	219-514-3
EC name:	1,3,5-tris(oxiranylmethyl) -1,3,5-triazine-2,4,6(1H,3H,5H) -trione
CAS number (in the EC inventory):	2451-62-9
CAS number:	414867-60-0
CAS name:	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-tris(2- oxiranylmethyl)- (a combination of α and ß isomers of TGIC)
IUPAC name:	1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione (a combination of α and ß-isomers)
Index number in Annex VI of the CLP Regulation	615-021-00-6
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 1,3,5-Triglycidyl-s-triazine-2,4,6-trione 1,3,5-Triglycidylhexahydro-1,3,5-triazine-2,4,6-trione 1,3,5-Triglycidylisocyanuric acid 1,3,5-Tris(2,3-epoxypropyl) isocyanurate 1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazine-2,4,6-trione 1,3,5-Tris(oxiranylmethyl)-1,3,5-triazine-2,4,6-trione Glycidyl isocyanurate N,N',N''-Triglycidyl isocyanurate NSC 269934 PTGIC TGT Triglycidyl isocyanurate Tris(2,3-epoxypropyl) isocyanurate Tris(epoxypropyl) isocyanurate TEPIC Araldite PT 810 TK 10622

Structural formula:



1.2 Composition of the substance

The currently available Registration Dossiers refer to a substance with the α : ß ration of ca. 90:10 but this dossier should cover all possible combinations of isomers, independent of their individual concentration in the substance covered by the CAS number 2451-62-9.

Name: 1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione

Description: epoxidized triazine

Degree of purity: Confidential information

Table 2: Constituents

Constituents	Typical concentration	Concentration range	Remarks
1,3,5-Tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione	Confidential information		

Table 3: Impurities

Impurities	Typical concentration	Concentration range	Remarks
Confidential information			

(Registration dossier 2011)

Table 4: Additives

Additives	Typical concentration	Concentration range	Remarks
Confidential information			

1.3 Physicochemical properties

Table 5: Overview of physicochemical properties

Property	Value	Reference
Physical state at 20°C and 101.3 kPa	Solid, which may occur as a white opaque powder or granules or as clear crystals	(Nordic Council of Ministers 2001)
Melting/freezing point	Melting point is 92 - 96 °C at normal pressure	**Value used for CSA in registration dossier:** 365 K at 1013 hPa Melting point was cited in NICNAS Chemical report (1994) from SDS data of data owner Ciba Geigy Ltd. The value measured with a particular batch was 92.8°C for TEPIC-G, and 94.6°C for Araldite PT810. Both values are within the range of 92 - 96°C published by NICNAS[1]
Boiling point	Boiling Point > 240°C	Boiling point not determined as decomposition occurred starting at ca. 240°C and higher
Vapour pressure	The vapor pressure of TGIC has been determined experimentally and by calculation. In the experimental study the value found was 0.0072 Pa at 20°C, and via calculation a lower value of 0.00091Pa (at 60°C) has been determined.	**Value used for CSA in registration dossier:** 0.007 Pa at 293.15 K The measured vapor pressure of 0.0072 Pa (20°C) is probably more reliable than the calculated one which is almost 10 –times lower at a higher temperature (60°C). A vapor pressure of 0.0072 Pa is however quite high when comparing it to another triazine - Atrazine which has a VP=0.00003853 Pa (at 20°C), or Simazine with a VP=0.000002946 Pa (at 20°C), or Cyanuric acid with a VP=0.0000040 Pa (at 20°C). This means that the real vapour pressure is probably at least one order of magnitude lower than the existing value, namely in the order of 0.0001 - 0.0007 Pa (at 20 °C)
Water solubility	Two values have been reported for the water solubility of 9000 mg/l	**Value used for CSA in registration dossier:** 9000

	and 10,000 mg/l, namely by NICNAS (1994), a value derived from NISSAN, and by Budnowsky (1968)	mg/L at 25 °C The water solubility is relatively high in destilled water, but the solubility drops fast with increasing salt concentrations as used for environmental studies. The water solubility of 9000 mg/l has been confirmed by Ciba Specialty Chemicals Inc in 1996.
Partition coefficient n-octanol/water (log value)	The log Pow cited by NICNAS (1994) originates from NISSAN, and is a measured value (-0.8, at 20°C).	**Value used for CSA in registration dossier:** Log Kow (Pow): -0.8 at 20 °C QSAR value of ECOSAR coincides well with the measured value of -0.8
Dissociation constant	pKa is not applicable for TGIC	TGIC has no functional groups to dissociate, it remains in water as parent molecule or is hydrolyzed, depending on pH.
Thermal stability	Indirect photolysis half-life = 26 - 73 days in water Photo-oxidation half-life in sunlight and air is 7 hours. Thermal stability is guaranteed up to 70 °C for a short period of time, No data on metal compatibility are available.	Photooxidation in sunlight and air is 7 hours. Thus, in air and under UV-light influence TGIC is not stable. Thermal stability is guaranteed up to 70 °C for a short period of time, No data on metal compatibility are available.

2 Harmonised classification and labelling

1,3,5-tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione is covered by Index number 615-021-00-6 in Annex VI, part 3 of Regulation (EC) No 1272/2008 as follows:

Table 6: 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)
615-021-00-6	1,3,5-tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione	Muta. 1B Acute Tox. 3 Acute Tox. 3 STOT RE 2 Eye Dam. 1 Skin Sens. 1 Aquatic Chronic 3	H340 H331 H301 H373 H318 H317 H412	GHS06 GHS08 GHS05 Dgr	H340 H331 H301 H373 H318 H317 H412

*Hazard class + category	Hazard statement code text
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)
Aquatic Chronic 3	H412 (Harmful to aquatic life with long lasting effects)

**** Hazard statement code:**
H317: May cause an allergic skin reaction. (by the oral and inhalation route)
H412: Harmful to aquatic life with long lasting effects
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 7: 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
615-021-00-6	1,3,5-tris(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione	Muta. Cat. 2; R46 T; R23/25 Xn; R48/22 Xi; R41 R43 R52-53	T R: 46, 23/25, 41, 43, 48/22, 52/53, S:53, 45, 61

*__Classification:__

Muta. Cat. 2;	R46	May cause heritable genetic damage.
T;	R23/25:	Toxic, Toxic by inhalation and if swallowed.
Xn;	R48/22:	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
	R52-53:	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

** **Labelling:**

T – toxic

R41 - risk of serious damage to eyes

R23/25 - toxic by inhalation and if swallowed

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

R52/53 - harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment

S45 - in case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

S61 - Avoid release to the environment. Refer to special instructions/Safety data sheets

S53 - avoid exposure - obtain special instructions before use

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(oxiran-2-ylmethyl)-1,3,5-triazinane-2,4,6-trione (EC number: 219-514-3, CAS number: 2451-62-9) is covered by index number 615-021-00-6 of Regulation (EC) No. 1272/2008 in Annex VI, part 3, Table 3.1 (the list of harmonised classification and labelling of hazardous substances) as mutagenic, Muta. 1B (H340; May cause genetic defects). The corresponding classification in Annex VI, part 3, Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 is mutagenic, Muta. Cat. 2, (R46; May cause genetic defects").

Therefore, this classification of the substance in Regulation (EC) No 1272/2008 shows 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, CHEMICAL SAFETY REPORT, 1,3,5-tris(oxiranylmethyl) -1,3,5-triazine-2,4,6(1H,3H,5H)–trione, EC Number: 219-514-3, CAS Number: 2451-62-9

Annex 1: Relevant Human Health Endpoints

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

No information of 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).

Together with other repeated dose studies, the following toxico-kinetic picture of TGIC can be drawn:

- 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 TGIC (a combination of 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 isomer combination 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 8: 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 µg/ml) in the first and second (0.47 - 30 µg/ml) test. TGIC without S9 (0.175, 0.35, 0.7, 1.4, and 2.8 µg/ml) TGIC with S9 (0.375, 0.75, 1.5, 3.0, and 6.0 µg/ml) method according to Clive, D. et al Validation and characterization of the L5178Y/TK+/-	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 without;	2 (reliable with restrictions) key study experimental result	P. Beilstein & D. Müller. (1983)

Method	Results	Remarks	Reference
mouse lymphoma mutagen assay system. Mutation Res. 59, 61-108 (1979)	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 OECD Guideline 482 (Genetic Toxicology: DNA	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
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 9: 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 mg/kg (nominal conc.) The methods applied in this study are referenced as follows: Boller, K. & Schmid, W.	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
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 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	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
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 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 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 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 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 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.

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