Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate

EC Number: 202-112-7
CAS Number: 91-97-4

CLH-O-0000006965-60-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

18 March 2021

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

3,3'-Dimethylbiphenyl-4,4'-diyl diisocyanate;

[TODI]

EC Number: **202-112-7**

CAS Number: **91-97-4**

Index Number: **n.a.**

Contact details for dossier submitter:

ANSES (on behalf of the French MSCA)
14 rue Pierre Marie Curie
F-94701 Maisons-Alfort Cedex
classification.clp@anses.fr

BAuA
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
44149 Dortmund, Germany

Version number: **2.0**

Date: **February 2020**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3,3'-DIMETHYLBIPHENYL-4,4'-DIYL DIISOCYANATE

CONTENTS

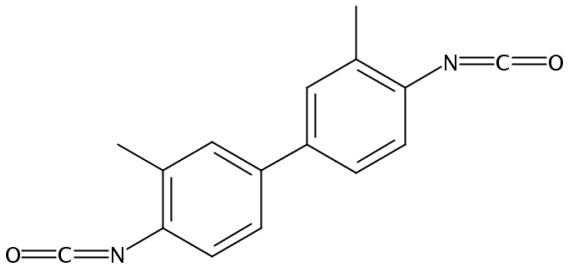
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3,3'-DIMETHYLBIPHENYL-4,4'-DIYL DIISOCYANATE

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3,3'-DIMETHYLBIPHENYL-4,4'-DIYL DIISOCYANATE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4,4'-Diisocyanato-3,3'-dimethylbiphenyl
Other names (usual name, trade name, abbreviation)	1-Isocyanic acid, 3,3'-dimethyl-4,4'-biphenylylene ester 1,1'-Biphenyl, 4,4'-diisocyanato-3,3'-dimethyl- 4,4'-Diisocyanato-3,3'-dimethyl-1,1'-biphenyl 3,3'-Bitolylene-4,4'-diisocyanate 3,3'-Dimethyl-4,4'-biphenylene diisocyanate 1-isocyanato-4-(4-isocyanato-3-methyl-phenyl)-2-methyl-benzene 1-isocyanato-4-(4-isocyanato-3-methylphenyl)-2-methylbenzene o-Tolidine diisocyanate TODI
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	202-112-7
EC name (if available and appropriate)	3,3'-Dimethylbiphenyl-4,4'-diyl diisocyanate
CAS number (if available)	91-97-4
Other identity code (if available)	-
Molecular formula	$C_{16}H_{12}N_2O_2$
Structural formula	
SMILES notation (if available)	Cc1cc(ccc1N=C=O)c2ccc(N=C=O)c(C)c2
Molecular weight or molecular weight range	264.28 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.1 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)
3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate EC No. 202-112-7 CAS No. 91-97-4	80-100	-	Acute Tox. 4 (H302/H312/ H332), Skin Irrit. 2 (H315), Eye Irrit. 2 (H319), Skin Sens. 1A/1 (H317), Resp. Sens. 1 (H334),

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3,3'-DIMETHYLBIPHENYL-4,4'-DIYL DIISOCYANATE

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self- classification and labelling (CLP)
			Muta 2 (H341), Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Current, proposed, and resulting harmonised classification and labelling for TODI

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate; [TODI]	202-112-7	91-97-4	Resp. Sens. 1 Skin Sens. 1A Carc. 1B	H334 H317 H350	GHS08 Dgr	H334 H317 H350			
Resulting Annex VI entry if agreed by RAC and COM											

Table 4: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route		
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation		
Serious eye damage/eye irritation		
Respiratory sensitisation	Harmonised classification proposed	Yes
Skin sensitisation		
Germ cell mutagenicity	Harmonised classification proposed	Yes
Carcinogenicity		
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure		
Aspiration hazard		
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

NOTE: This dossier is the result of the combined efforts of ANSES (FR) and BAuA (DE). ANSES prepared the sections on Germ cell mutagenicity and Carcinogenicity and will be responsible at a later stage for replying to any potential comments arising from the Consultation on those hazard

classes. BAuA (DE) prepared the sections on Respiratory and skin sensitisation and will be the responsible party for addressing comments on those sections.

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Not applicable

RAC general comment
3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate (TODI) is a substance used for the manufacture of plastic products and has no current entry in Annex VI to the CLP regulation.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

According to Article 36 of the CLP regulation, respiratory sensitisation is an endpoint for which Harmonised Classification and Labelling (CLH) is warranted. Although skin sensitisation is not covered by Article 36, there is a close relationship between skin sensitisers and respiratory sensitisers (currently all known low molecular weight chemical respiratory sensitisers are also skin sensitisers). Therefore, it is the view of the Dossier Submitter (DS) that an assessment of skin sensitisation potential is an integral part of the assessment of respiratory sensitisation.

According to Article 36 of the CLP regulation, mutagenicity and carcinogenicity are endpoints for which Harmonised Classification and Labelling (CLH) is warranted. Therefore, no justification is needed.

5 IDENTIFIED USES

A summary of the information available on ECHA's public website (accessed 2017-12-17) is given below[1].

5.1 General

This substance is manufactured and/or imported in the European Economic Area in 10 - 100 tonnes per year. This substance is used in articles and at industrial sites.

5.2 Consumer Uses

ECHA has no public registered data indicating whether or in which chemical products the substance might be used. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment.

5.3 Article service life

This substance is used in the following activities or processes at workplace: The low energy manipulation of substances bound in materials or articles and manual maintenance (cleaning and repair) of machinery. Other release to the environment of this substance is likely to occur from: outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials) and indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products, electronic equipment). This substance can be found in products with material based on: plastic.

[1] The text is a mixture of excerpts from ECHA's public website and of text prepared by the DS. Direct use of original text is not specifically marked.

5.4 Widespread use by professional workers

ECHA has no public registered data indicating whether or in which chemical products the substance might be used. ECHA has no public registered data on the types of manufacture using this substance. ECHA has no public registered data on the use of this substance in activities or processes at the workplace. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment.

5.5 Formulation or re-packing

ECHA has no public registered data indicating whether or in which chemical products the substance might be used. ECHA has no public registered data on the use of this substance in activities or processes at the workplace. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment.

5.6 Uses at industrial sites

ECHA has no public registered data indicating whether or in which chemical products the substance might be used. This substance is used for the manufacture of: plastic products. This substance is used in the following activities or processes at workplace: Closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, transfer of chemicals at dedicated facilities, laboratory work, production of mixtures or articles by tabletting, compression, extrusion or pelletisation and the low energy manipulation of substances bound in materials or articles. Release to the environment of this substance can occur from industrial use: in the production of articles, as an intermediate step in further manufacturing of another substance (use of intermediates) and for thermoplastic manufacture.

5.7 Manufacture

ECHA has no public registered data on the use of this substance in activities or processes at the workplace. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment.

6 DATA SOURCES

This report has been created based on the data submitted by the lead registrant in the REACH registration dossier for TODI. In addition, further relevant data on TODI and related diisocyanates were retrieved as part of a general literature search in the context of the restriction proposal for diisocyanates recently submitted to ECHA by DE.

A supplementary literature search was performed in the SCOPUS database on 2017-06-30 for all references in the areas of medicine, pharmacology, toxicology, or environment published in 2015-2017 and containing the keyword „isocyanate". Also the PubMed database was searched for that keyword and time range.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties (all data taken from REACH registration dossier)

Property	Value	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Sensory determination [EPA OPPTS 830.6303 (Physical State)]
Melting/freezing point	Melting point: 71.7 °C (at 101.29 kPa)	Experimental result [OECD Guideline 102 (Melting point / Melting Range): differential scanning calorimetry]
Boiling point	Decomposition at approximately 644 K (371°C) at 101.42 kPa before boiling	Experimental result [EU Method A.2 (Boiling Temperature): differential scanning calorimetry)]

<table>
<tr><th>Property</th><th>Value</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Relative density</td><td>1.331 (at 20°C)</td><td>Experimental result
[OECD Guideline 109 (Density of Liquids and Solids): air comparison pycnometer (for solids)]</td></tr>
<tr><td>Vapour pressure</td><td>0.0029 Pa (at 25 °C)</td><td>Calculated value
[QSAR (MPBPWIN v1.43: Modifiied Grain Method)]
This value is confirmed by measured vapor pressure of MDI (structurally close molecule).</td></tr>
<tr><td>Surface tension</td><td>Determination of surface tension for TODI is scientifically not feasible.
The substance is hydrolytically unstable at pH 4, 7 and 9 (half-life less than 1 min).</td><td>-</td></tr>
<tr><td>Water solubility</td><td colspan="2">Determination of water solubility for TODI is scientifically not feasible.
The substance is hydrolytically unstable at pH 4, 7 and 9 (half-life less than 12 hours).</td></tr>
<tr><td>Water solubility, ctd.</td><td>Water solubility of the hydrolysis degradation product 4,4'-bi-o-toluidine (TODA):
1.3 g/L (at 25°C)</td><td>Handbook data
[CRC Handbook of Chemistry and Physics, 88th edition, 15. June 2007]</td></tr>
<tr><td rowspan="2">Partition coefficient n-octanol/water</td><td>Determination of partition coefficient of TODI is scientifically not feasible.
Given TODI high reactivity with water (half-life of TODI in water < 1min) and other protonic solvent (octanol), partition coefficient is not relevant and this property doesn't need to be assessed for isocyanate molecules.</td><td></td></tr>
<tr><td>Calculated log Kow of the hydrolysis degradation product 4,4'-bi-o-toluidine (TODA): 3.0176</td><td>Calculated value
[QSAR (EPIWIN using KOWWIN v1.68)]</td></tr>
<tr><td>Granulometry</td><td><table><tr><th>Sieve size [µm]</th><th>Distribution</th></tr><tr><td>63</td><td>0.0 %</td></tr><tr><td>125</td><td>0.2 %</td></tr><tr><td>250</td><td>0.6 %</td></tr><tr><td>500</td><td>4.3 %</td></tr><tr><td>1000</td><td>24.2 %</td></tr><tr><td>2000</td><td>70.6 %</td></tr><tr><td>4000</td><td>0.0 %</td></tr></table>
A range of 2000 to 1000 µm covers 90 % of the particle size distribution of TODI. No particles with a diameter below 63 µm were found.</td><td>Experimental result
[CIPAC MT 170 Dry Sieve Analysis of Water Dispersible Granules;
mass distribution: machine sieving]</td></tr>
<tr><td>Stability in organic solvents and identity of relevant</td><td>N.a. (stability in organic solvents is not a critical property of the substance)</td><td>-</td></tr>
</table>

Property	Value	Comment (e.g. measured or estimated)
degradation products		
Dissociation constant	N.a. (hydrolytically unstable)	-
	pKa of the hydrolysis product 4,4'-bi-o-toluidine (TODA): 4.59 (at 25 °C)	Calculated value [QSAR (Advanced Chemistry Development (ACD/Labs) Software V11.02 (1994-2013))]
Viscosity	N.a. (solid)	-

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

To the best knowledge of the DS, no studies on the ADME properties of TODI in mammals are available. To justify this, the lead registrant refers to the high and rapid reactivity of TODI with water. A hydrolysis test was performed at 50 ± 0.5 °C and 25 ± 2 °C, at pH 4, 7 and 9 which is summarised by the lead registrant as follows:

One sample was analysed at each time point. For each assay, the first 'start' time point for hydrolysis was made as quickly as the sample could be loaded into the HPLC system and analysed (typically 3 to 5 minutes). At the first time point and all subsequent time points at pH 4 and 9 at 50 ± 0.5 °C, the maximal amount of hydrolysis product was measured, hence the $t_{1/2}$ was less than at the first measurement time point. At 25 °C, and pH 9 full hydrolysis was within 30 minutes with 50% at the 'start' time point; at 25 °C, pH 4 full hydrolysis was found at the first time point. It is concluded that TODI hydrolysed rapidly (in less than 30 minutes) at 25 and 50 °C at pH 4 and 9. At pH 7 hydrolysis of 100% was reached within 29 hours (25°C) and 2.5 hours (50°C). For the tests carried out at pH 7 the log-transformed data of peak areas against time were plotted. A line was fitted on the measured data and the rate constant and the half-life were obtained from its slope according to equations 2 and 3. The t1/2 at pH 4 and 9, at 25 and 50 °C was lower than or equal to 2 minutes; at pH 7 the $t_{1/2}$ was 16 hours and 1.2 hours at 25 and 50 °C respectively (Laky, 2009).

While these data confirm the potential of TODI for fast hydrolysis, the DS nevertheless finds that upon contact with skin or the respiratory tract a sufficiently large time window is available for the initial steps of sensitisation to take place.

Furthermore the lead registrant has included an expert statement on the ADME properties of TODI in the REACH registration dossier (SCC, 2010), which however, essentially refers data for MDI without a closer analysis of commonalities or differences between the two substances. In the view of the DS, this statement does not include relevant ADME information with respect to (respiratory or skin) sensitisation.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not assessed in this dossier

10.2 Acute toxicity - dermal route

Not assessed in this dossier

10.3 Acute toxicity - inhalation route

Not assessed in this dossier

10.4 Skin corrosion/irritation

Not assessed in this dossier

10.5 Serious eye damage/eye irritation

Not assessed in this dossier

10.6 Respiratory sensitisation

10.6.1 Endpoint definition and evaluation strategy

According to Annex I, section 3.4.1.1 of the CLP regulation "respiratory sensitiser means a substance that will lead to hypersensitivity of the airways following inhalation of the substance" (European Parliament and Council, 2008).

Since there is still no validated and universally accepted test method for identifying respiratory sensitisers, there is currently no standard information requirement under REACH for this endpoint. For the most commercially successful diisocyanates on the market, such as HDI, MDI, or TDI, nevertheless a comprehensive database of human and non-human data is available demonstrating the potential of these substances to cause respiratory sensitisation (RS) in humans. In contrast, for those diisocyanates used in lower volumes such as TODI, the substance addressed by this dossier, data with respect to RS are scarce. For TODI, specifically, no human or animal data related to RS were identified by the DS.

Article 9 of the CLP regulation specifies how the hazard information is evaluated to decide on classification. The strategy followed in this dossier is therefore characterised by a category approach by means of which the knowledge about the RS potential of the three most commonly used diisocyanates HDI, MDI, and TDI is read across to TODI. The use of category-based read-across for classification and labelling is covered by Article 5 1. (2) of the CLP regulation, which in turn refers to the methods listed in section 1 of REACH Annex XI. The category approach is justified in the following section. Finally, all available information is combined in an overall weight-of-evidence assessment in line with CLP Annex I, section 1.1.1.3.

10.6.2 Justification of the category approach

10.6.2.1 Characterisation of the category approach in terms of the ECHA Read-Across Assessment Framework (RAAF, (ECHA, 2017b))

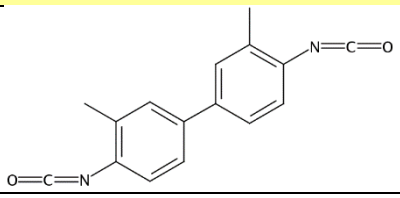
The approach relates to RAAF Scenario 6 (human health), i.e. the read-across hypothesis for the category is based on different compounds which have qualitatively similar properties, with no relevant variations in properties observed among source substances and the same strength predicted for the target substance[2].

The following sub-sections provide the justification for the read-across hypothesis, structured according to the Assessment Elements (AE) relevant for Scenario 6, as listed in Appendix F to the RAAF.

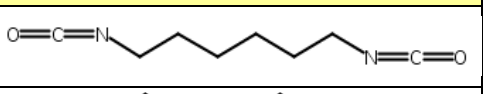
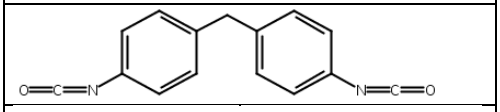
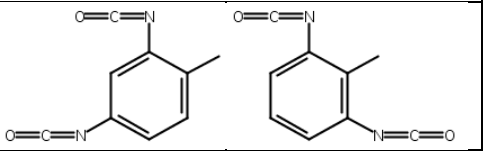
10.6.2.2 AE C.1 Substance characterisation

The identity of the target substance TODI has been characterised above. Table 6 provides information on the identity and harmonised classification of the target substance as well as the category source substances HDI, MDI, and TDI.

Table 6: List of category source substances used for read-across to TODI

EC Name; trivial name used in this report	EC No. CAS no.	CLH for sensitisation (Annex VI to CLP)	Structure
3,3'-Dimethylbiphenyl-4,4'-diyl diisocyanate; TODI	202-112-7 91-97-4	-	

[2] Note that here the terms "no relevant variations" and "same strength" relate to the question "respiratory sensitiser – yes or no?" and not to relative potency.

EC Name; trivial name used in this report	EC No. CAS no.	CLH for sensitisation (Annex VI to CLP)	Structure
Hexamethylene diisocyanate; HDI	212-485-8 822-06-0	Resp. Sens. 1 Skin Sens. 1	
4,4'-Methylenediphenyl diisocyanate; MDI[$]	202-966-0 101-68-8		
m-Tolylidene diisocyanate (80/20 mixture of 2,4-TDI and 2,6-TDI isomers); TDI[$]	247-722-4 26471-62-5		

[$] The DS is aware that there are other isomers or isomer mixtures of MDI and TDI, but in this report these abbreviations refer only to the isomers listed in this table.

10.6.2.3 AE C.2 Structural similarity and category hypothesis

As can be seen in Table 6, all members of the group (as well as the target substance) are monomeric diisocyanates, i.e. they share the structural feature of two isocyanate functional groups. The part of the molecular structure linking the two isocyanate groups may be variable.

10.6.2.4 AE C.3 Link of structural similarities and structural differences with the proposed regular pattern

It will be illustrated in the following sections that the respiratory sensitisation property depends solely on the diisocyanate feature common to sources and target, independent of variations in the molecular structure connecting the two isocyanate groups.

10.6.2.5 AE C.4 Consistency of effects in the data matrix

For all three source substances, plenty of human and non-human data are available to consistently demonstrate their potential to cause RS (cf. section below). Consequently, all three congeners share harmonised classification as Resp. Sens. 1. For details, the reader is referred to sections 10.6.4 and 10.6.5 as well as to Annex I.

10.6.2.6 AE C.6 Reliability and adequacy of the source data

This is addressed in the relevant parts of sections 10.6.4 and 10.6.5 as well as in Annex I.

10.6.2.7 AE 6.1 Compounds the test organism is exposed to

In all studies used in this approach, the test organisms have been exposed to the source substances as described in Table 6 above.

10.6.2.8 AE 6.2/6.3 Common underlying mechanism, qualitative/quantitative aspects

In 2012, the Organisation for Economic Co-Operation and Development (OECD) published the Adverse Outcome Pathway (AOP) for skin sensitisation initiated by covalent binding to proteins (OECD, 2012). Enoch and co-workers hypothesised that in a similar way covalent binding of electrophiles to proteins in the lung marks the molecular initiating event (MIE) in a putative AOP for RS. In several publications, the authors characterised the corresponding chemical reaction domains and identified structural alerts which have now been integrated as profilers into the OECD QSAR Toolbox (Enoch et al., 2011; Enoch et al., 2009; Enoch et al., 2014). According to the authors, *"iso(thio)cyanates have been shown to undergo an acylation reaction resulting in the formation of protein adducts"* (Enoch et al., 2011). This is also shown in Figure 1 below.

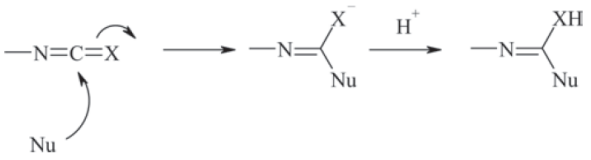


Figure 1: Acylation reaction for isocyanates (X = oxygen). Reproduced from (Enoch et al., 2011)

The isocyanate moiety is indeed a common alert in RS prediction tools. Dik et al. tested five different RS prediction models with a test chemical set also including isocyanates and diisocyanates; all of the models agreed on a positive prediction in all of the cases (Dik et al., 2014). In fact the IR & CSA guidance, chapter R.7a recommends to use the test set from this publication as a source for read-across (ECHA, 2016).

Agius et al. noted that *"low molecular weight agents that can form at least two bonds with native human macromolecules carry a higher occupational asthma hazard. Thus bi- or polyfunctional low molecular weight agents such as diisocyanates and aliphatic or cyclic amines, as well as dicarboxylic acid anhydrides and dialdehydes, rank highly among organic low molecular weight substances"* (Agius, 2000). A potential explanation might be found in that bifunctionality potentially allows for cross-linking of nucleophilic moieties within the same or different proteins which may result in a more marked change of conformation.

The potential reactivity of the diisocyanate source substances given in Table 6 above towards amino acids such as cysteine and lysine has been shown *in chemico* (Lalko et al., 2013).

In summary, the isocyanate functional group marks a well-known structural alert for RS for which there is some evidence that interaction with proteins might occur via an acylation type reaction between the electrophilic NCO functional group(s) and nucleophilic protein moieties such as amino or sulfhydryl groups.

Moreover, with respect to Table 6 above, DE would like to point out that in terms of structure those molecular parts of the source substances separating the two isocyanate groups differ from each other, further highlighting that at least qualitatively the presence of the (two) isocyanate groups is the decisive factor for the RS potential, while the remaining molecular structure is of less importance (it might however have an impact on the physico-chemical and ADME properties and therefore relative potency which are not addressed in this dossier).

10.6.2.9 AE 6.4 Exposure to other compounds than those linked to the prediction

DE is not aware that the presence of other compounds has influenced the outcome of the studies used for the category approach.

10.6.2.10 AE C.6 Bias that influences the prediction

Only the three most commonly used diisocyanates have been used as source substances, because most published literature on diisocyanates relates to these compounds. However, DE notes that a number of further diisocyanates share classification as RS. An overview is given in the recent restriction report for diisocyanates (German CA, 2016) and the associated annex. DE is not aware of any monomeric diisocyanate for which data convincingly show that the substance is not a respiratory (and skin) sensitiser.

10.6.3 Data retrieval, evaluation, and presentation strategy

Based on the above considerations, the strategy for data research and presentation followed in this dossier was chosen by DE as follows:

- Identify all studies in humans and animals for TODI, HDI, MDI, and TDI. Notably, numerous studies demonstrate the ability of diisocyanates to cause symptoms of RS also after dermal exposure (cf. the restriction report for diisocyanates recently submitted by the German MSCA[3]), however, since the definition from the CLP regulation cited in section 10.6.1 clearly asks for inhalation exposure, only studies along this route were evaluated for the current dossier.
- Evaluate and present the relevant human data for the three source substances HDI, MDI, and TDI (no relevant studies were identified for TODI).
- Filter animal data for relevance according to predefined criteria (cf. section 10.6.5).
- Evaluate and present the relevant animal data for the three source substances HDI, MDI, and TDI (no relevant studies were identified for TODI).

[3] https://echa.europa.eu/registry-of-submitted-restriction-proposal-intentions/-/substance-rev/15016/term, last accessed 2017-10-21

- Summarise, compare to the CLP criteria and conclude on a possible potential for RS.

10.6.4 Human data

The CLP regulation notes that evidence for chemical-induced RS (asthma/rhinitis/conjunctivitis/alveolitis) will normally be based on human experience. "*The condition will have the clinical character of an allergic reaction. However, immunological mechanisms do not have to be demonstrated"* (European Parliament and Council, 2008).

Human data relevant for RS assessment may comprise *"consumer experience and comments, preferably followed up by professionals (e.g. bronchial provocation tests, skin prick tests and measurements of specific IgE serum levels); records of workers' experience, accidents, and exposure studies including medical surveillance; case reports in the general scientific and medical literature; consumer tests (monitoring by questionnaire and/or medical surveillance); epidemiological studies."* (ECHA, 2016).

Both immediate (seconds to minutes) and late-onset (up to several hours) hypersensitivity reactions may be present in patients with diisocyanate-induced asthma, with the prevalence of late responses being as high as 70% (Niimi et al., 1996). The delay between onset of (low-level) exposure at work and the manifestation of the asthmatic symptoms, which may be as long as several years after the start of exposure, is of particular concern. In addition, patients often develop persistent bronchial hyperresponsiveness (BHR; often also the more general term "airway hyperresponsiveness/hyperreagibility (AHR)" is used interchangeably) to non-specific stressors including e.g. other chemicals such as methacholine, cold, dust, or physical exercise that can last for years even in the absence of continued exposure, and complete recovery of lung function may never be achieved (Johnson et al., 2004a).

The following endpoints are used regularly for the diagnosis of occupational asthma in human case reports, case studies, and epidemiological studies:

- clinical symptoms: wheezing, dry cough, intermittent shortness of breath, particularly in connection with physical activity,
- lung function testing following unspecific or specific bronchial provocation: Forced Expiratory Volume in one second (FEV_1), Peak Expiratory Flow (PEF), and
- presence of diisocyanate-specific IgE and/or IgG antibodies.

Nevertheless, studies in humans frequently suffer from limitations. The full spectrum of parameters such as the test protocol used, the substance or preparation studied, the extent of exposure, the frequency of effects, the persistence or absence of health effects, the presence of confounding factors, the relevance with respect to group size, statistics, documentation, or the "healthy worker effect" which should all be reported (ECHA, 2016), is rarely, if ever, provided in these reports.

10.6.4.1 Human data for the target substance TODI

No relevant data for TODI were identified during the literature search performed for this dossier.

10.6.4.2 Human data for the source substances HDI, MDI, and TDI

More than 100 case reports and epidemiological studies have been evaluated. An overview of this evaluation is provided in Annex I, Table 1 (case reports) and Tables 2-7 (epidemiological studies). The case reports provide overwhelming proof that humans exposed to the source substances HDI, MDI, and/or TDI may suffer from a broad spectrum of respiratory effects including asthma and pathological changes of the airways. Also a number of fatal cases have been reported, albeit not in recent years. While during the early stages of the development of the disease, respiratory symptoms may eventually be reversed upon removal from exposure, an irreversible remodelling of the airways will eventually take place when exposure is continued. On the other hand these case reports do not allow for an assessment of the frequency of occurrence of respiratory sensitisation to TODI in the human population as they feature only a small number of patients and it is not known which fraction of all exposed persons is affected (and which fraction of the affected is reported). They

are therefore not suited for sub-categorisation. In addition, no harmonised approach for sub-categorising respiratory sensitisers is available yet.

An overview of epidemiological studies on diisocyanates and respiratory effects conducted until today with short study descriptions and results is given in Annex 1, Tables 2-7. Despite a large number of available studies, none of these studies is eligible for deriving a reliable Exposure-Response-Relationship (ERR) due to limitations of the studies. This is also inherent in the mechanism of the disease. No study overcomes the problem that sensitive predictive markers for diisocyanate sensitisation are missing and that dermal exposure as well as inhalation peak exposure likely contribute to the induction of sensitisation, but cannot be assessed appropriately to date.

10.6.5 Animal data

The recent update of the IR & CSA guidance, section R.7a notes that *"although predictive models are under validation, there is as yet no internationally recognised animal method for identification of respiratory sensitisation."* (ECHA, 2016).

In concert with human data, some types of animal data may play a supportive role in the qualitative assertion of respiratory sensitisation (ECHA, 2016; ECHA, 2017a; European Parliament and Council, 2008). With respect to the nature of relevant animal data, the CLP regulation states that *"data from appropriate animal studies which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans may include: (a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice; (b) specific pulmonary responses in guinea pigs"*(European Parliament and Council, 2008).

From this wording the DS concludes that (test substance-specific) changes in immunological parameters as well as specific pulmonary responses may be important indicators of RS, whereas the absence of such effects in animals cannot serve as a proof of the absence of RS potential in humans. With respect to the species named in the regulation, over the years various animal species have been used as model species for RS and to the knowledge of the DS there is no scientific argument why immunological changes should only be relevant in mice or pulmonary responses only relevant in guinea pigs.

As a consequence, the animal database available for the three source substances and the target substance TODI has been evaluated and filtered for relevant studies (the complete list of studies is available in Table 8 in Annex I to this dossier). To that end, studies were discarded which used induction routes other than the inhalation route (or mixed designs including e.g. intradermal and inhalation induction). Only true inhalation studies were accepted, while those using intranasal exposure, intratracheal instillation, or oropharyngeal administration were not considered any further.

In the next step, studies were considered unreliable and therefore excluded from assessment if any of the following information was missing or incomplete:

- identity of the test substance
- the physical state of the test substance as applied (aerosol or vapour),
- the inhalation protocol followed (whole-body or head-/nose-only),
- confirmation of the presence of a negative control, and
- the number of animals per dose group.

Animal study designs for respiratory sensitisation have been manifold, involving a variety of species, protocols, and target endpoints, and a standardised protocol with regulatory acceptance is still missing. Therefore a negative result from an animal experiment on RS is not suitable to exclude the need for classification and labelling. Consequently, for the read-across assessment the evaluation concentrated on data providing a positive indication of respiratory sensitisation, therefore for HDI, MDI, and TDI only studies reporting the presence of one or more relevant effects were selected for further processing. Where several experiments were reported in one study report, only those with effects were processed further. Finally, studies using agents other than TODI or the three source substances (as per Table 6) in their monomeric form, i.e. their prepolymers, breakdown products or protein conjugates or other isomers for induction, or for which the exact identity was unclear, were also dismissed.

The effects observed in the remaining studies were captured according to the following four categories (and the experiments included or dismissed accordingly):

- production of test substance-specific IgE and/or IgG antibodies; for this, also experiments without an elicitation/challenge elicitation step were included,
- elicitation of dermal contact hypersensitivity (positive results in skin sensitisation tests upon intradermal or topical challenge); in the view of DE, such experiments would also provide proof of a substance-specific immunological reaction. In the same sense, two reports of a "respiratory LLNA", i.e. an evaluation of the draining mandibular lymph nodes after inhalation induction by means of a stimulation index analogous to that used in the dermal LLNA, were included,
- impact on respiratory function; experiments showing effects on respiratory function were only included if these effects occurred as the result of a test substance-specific challenge, after repeated exposure, or after continuous exposure for several days. The latter two cases were included since the immune response will develop in parallel to repeated/continuous exposure and therefore later exposures or a later stage of long-time continuous exposure will have the character of an elicitation/challenge more than of an induction exposure. For their relevance in human asthma diagnostics, also animal experiments employing unspecific challenges (e.g. with methacholine) to demonstrate AHR were included, although the CLP criteria ask for "specific pulmonary reactions" (cf. above). A decrease instead of an increase in respiratory rate was attributed to sensory irritation and experiments showing only this effect were excluded from further evaluation (although from a linguistical point of view, this would also constitute a "specific pulmonary reaction"),
- presence of inflammation markers (e.g. seen in histopathological evaluations or found in bronchoalveolar lavage fluid); to delineate RS from mere irritation, studies were only included if a) more than one exposure or a continuous exposure over more than one day occurred and b) at least one effect from any of the other three categories was found in the same study (not necessarily the same experiment).

In the end, a total of 36 experiments from 18 study reports, performed in guinea pigs, mice, and rats qualified for further evaluation. Table 7 provides an overview of the number of studies and their distribution over the different substances and rodent species.

Table 7: Overview of the number of available animal experiments per substance and species

Diisocyanate	Species			Total
	Guinea pigs	Mice	Rats	
TODI	-	-	-	-
HDI	-	3	-	3
MDI	6	-	6	12
TDI	14	7	-	21
Total	20	10	6	36

10.6.5.1 Animal data for the target substance TODI

For TODI, no relevant animal studies/experiments with inhalation exposure were identified during the literature search for this dossier.

10.6.5.2 Animal data for the source substances HDI, MDI, and TDI

Table 8 provides an overview of the results of the experiments with HDI, MDI, and TDI selected for further evaluation regarding the potential of these substances to cause respiratory sensitisation.

Table 8: Studies for evaluating the potential of the source substances HDI, MDI, and TDI to cause RS in rodents following exposure via the inhalation route (sorted by species and year, see section 0 for abbreviations)

Strain	Sex	"Induction" Agent	"Elicitation" Route	"Elicitation" Agent	Physical state	Inhalation type	Animals/group	No. of "induction"	Hours/exposure	Total days	Critical effect	Reference
Guinea pigs												
ESH	F	TDI	-	-	VP	HO	8	2	3	3	AB	(Karol, 1983)
							12	5		5	SS	
			IDE	TDI-GPSA			8					
			INH	TDI-GPSA/ TMI-GPSA			12				RF	
DH	F	TDI	INH	TDI-GPSA	AE	NO	10	5	3	5	AB/RF	(Botham et al., 1988)
DH	F	MDI	-	-	VP	NO	5	5	3	21	AB	(Dearman and Botham, 1990)
			IPE	MDI-GPSA						22		
Hartley	F	TDI	INH	TDI	VP	WB	7	5	3	21	AB/IF/RF	(Huang et al., 1993a)
Hartley	F	TDI	INH	TDI	VP	WB	6	5	3	26	AB/RF	(Aoyama et al., 1994)
Hartley	?	MDI	INH	MDI	AE	NO	≥ 8	1	0.25	21/ 22	RF	(Pauluhn, 1994)
				MDI-GPSA								
		TDI		TDI	VP							
				TDI-GPSA								
DH	F	MDI	INH	MDI	AE	NO	16	5	3	18	AB	(Rattray et al., 1994)
?	?	MDI	INH	MDI	AE	NO	16	1	0.25	21/ 28	AB/RF	IUCL: (Bayer, 1995)
DH	F	TDI	-	-	VP	WB	20	1	48	3	RF	(Gagnaire et al., 1996)
									168	8		
DH	F	TDI	-	-	VP	WB	10	1	134 4	56	RF	(Gagnaire et al., 1997)
DH	F	TDI	INH	TDI/TDI-GPSA	VP	NO	8	1	0.25	21	AB/IF/RF	(Pauluhn and Mohr, 1998)
Hartley	F	TDI	TOP	TDI	AE	NO	8	1	4	15	SS	(Ebino et al., 2001)
Mice												
C57BL/6	F	TDI	INH	TDI	VP	NO	5	30	4	56	AB/IF/RF	(Matheson et al., 2005a)
C57BL/6	F	TDI	INH	TDI	VP	HO	5	1	2	1	AB/IF/RF	(Matheson et al., 2005b)
								30	4	56		
BALB/c	F	TDI	INH	TDI	VP	WB	6-8	1	4	14	AB/IF	(Ban et al., 2006)
BALB/c	M	HDI	-	-	VP	NO	6	3	0.75	5	IF	(Arts et al., 2008; de Jong et al., 2009)
									1.5			
									3			
		TDI							0.75			
									1.5			
									3			
Rats												
Wistar	F	MDI	-	-	AE	WB	8	436	17	610	RF	IUCL: (Hoymann et al., 1995)
							12				IF	
							20	65		98		
								260		365		
								436		371		
							80	520		728		

10.6.5.2.1 Guinea pigs

After exposing female English Smooth-Hair guinea pigs to vapour containing 0.02 ppm TDI twice for 3 h/d within 3 days, Karol demonstrated an increased production of TDI-specific antibodies. After five 3 h/d exposures on 5 consecutive days at concentrations of ≥ 0.12 ppm TDI, again specific antibodies were found (at concentrations ≥ 0.36 ppm); moreover, contact hypersensitivity was observed as a result of intradermal challenge with TDI-guinea pig serum albumin conjugate (TDI-GPSA) at concentrations of ≥ 0.12 ppm. Finally, following a specific bronchial provocation challenge with TDI-GPSA, a significant increase in respiratory rate (RR) was reported at ≥ 0.36 ppm (Karol, 1983).

Botham et al. (1988) reported the production of TDI-specific IgE- and IgG_1 antibodies as well as an increase in RR after bronchial provocation challenge with TDI-GPSA following exposure of female Dunkin-Hartley guinea pigs to 1, 3 or 4 ppm TDI for 3 h/d on five consecutive days (Botham et al., 1988). In 1990, Dearman and Botham used the same exposure protocol in female Hartley guinea pigs with 11 mg/m^3 MDI vapour and found an increased production of specific IgG_1 and – to a lesser degree – IgE antibodies. Intraperitoneal challenge with MDI-GPSA diminished the IgE, but not the IgG response (Dearman and Botham, 1990).

Huang et al. demonstrated increased histamine blood levels as well as mast cell degranulation indices at concentrations ≥ 0.12 ppm TDI after exposing female Hartley guinea pigs to TDI concentrations ranging from 0.03 to 0.37 ppm for 3 h/d over 5 d and challenging them with TDI three weeks later (Huang et al., 1993b). In 1994, the same group used a similar design (with induction concentrations of ≥ 0.02 ppm TDI) and demonstrated formation of TDI-specific IgG antibodies as well as effects on respiratory function (as percentage increase in respiratory rate) at concentrations ≥ 0.2 ppm (Aoyama et al., 1994).

Pauluhn sensitised guinea pigs via inhalation by a single 15 min exposure to 135 mg MDI/m^3 or to 45 mg TDI/m^3. Upon challenge with the same diisocyanate, either unbound or conjugated to GPSA at approximate concentrations of 12 (MDI) or 4 mg/m^3, 21 d post-induction, increased immediate onset responses in respiratory function (in terms of a dimensionless parameter composed of peak expiratory flow rate, inspiratory and expiratory time/volume and tidal volume) vs. ovalbumin (OVA) controls were observed. The same animals displayed increased acetyl provocation indices vs. OVA when subjected to an acetylcholine provocation test one day later, i.e. 22 d post-induction (Pauluhn, 1994).

Rattray and co-workers reported a slight increase in IgG_1 levels in female Dunkin-Hartley guinea pigs 18 d after five 3 h/d exposures to atmospheres containing ca. 20 mg MDI/m^3 (Rattray et al., 1994).

In another study in guinea pigs, the animals were exposed via inhalation to 132 mg MDI aerosol/m^3 for 20 min. Depending on the test group, challenge by inhalation was performed 21 or 28 days later, using a ramped test design (increasing concentrations of 0/5/15/35 mg MDI/m^3, successively for 20 min per concentration level resulting in a total MDI exposure time of 1 h). According to the authors of the IUCLID summary, *"low anti-MDI antibody titers* [were observed] *in animals sensitized to MDI (15/16). No association between elevated IgG1 anti-MDI antibody titers and respiratory responses or any of the bronchoalveolar lavage parameters could be established. […] Only a borderline sensitisation occurred […]. Mild MDI-specific immediate-onset responses were observed mainly during challenge to slightly irritant concentrations (35 mg/m³). A marked increase of neutrophilic or eosinophilic granulocytes could not be established. An activation of these cells could not be observed. Animals sensitized to high concentrations of aerosolized MDI showed a mild airway hypersensitivity without concomitant influx of inflammatory cells"* (Bayer, 1995).

Gagnaire and co-workers demonstrated the development of AHR/BHR (measured as the dose of acetylcholine in a bronchial provocation test required to cause a two-fold increase in airway resistance vs. baseline) in female Dunkin-Hartley guinea pigs following continuous exposure to 0.08 ppm TDI for 48 h, 0.046 ppm for one week, or 0.029 ppm for eight weeks (Gagnaire et al., 1997; Gagnaire et al., 1996).

Pauluhn and Mohr applied different inhalation exposure designs (1 x 15 min, 5 x 3 h/d, using different concentrations of 3.8 to 51 mg TDI/m^3) to test female Dunkin-Hartley guinea pigs for respiratory sensitisation. They noted AHR/BHR (measured as a "flow-derived dimensionless parameter", or "FDP") after challenge with acetylcholine (ca. on days 20 and 22), TDI (day 21) and TDI-GPSA hapten-protein complex (around day 28). Four weeks into the test, production of TDI-specific IgG_1 antibodies was demonstrated. On sacrifice one day after the conjugate challenge, inflammation markers and histopathological lesions in the airways were observed to a varying degree in all groups (Pauluhn and Mohr, 1998).

Ebino and co-workers demonstrated skin sensitisation upon topical TDI challenge of Hartley guinea pigs sensitised two weeks before by a single four hour inhalation exposure to TDI (Ebino et al., 2001).

10.6.5.2.2 Mice

In studies in C57BL/6 mice using a single, 1-h inhalation challenge following a 6 wk inhalation induction regime (4 h/d, 5 d/wk), Matheson and co-workers (2005) observed *"a marked allergic response evidenced by increases in airway inflammation, eosinophilia, goblet cell metaplasia, epithelial cell alterations, airway hyperresponsiveness (AHR), TH1/TH2 cytokine expression in the lung, elevated levels of serum IgE, and TDI-specific IgG antibodies, as well as the ability to transfer these pathologies to naïve mice with lymphocytes or sera from TDI exposed mice"* (Matheson et al., 2005a; Matheson et al., 2005b).

Ban and co-workers induced sensitisation in female BALB/c mice by 4 h-exposure via whole-body inhalation to 3 ppm TDI on three consecutive days[4]. Challenge was either performed by two single 4 h challenges with 0.3 ppm TDI 7 or 12 days after the end of induction or by a single 4 h inhalation challenge with 2 ppm TDI 14 days after the end of induction, followed by a 1 d tracheal instillation with 50 µg TDI-HAS conjugate/animal one week later. The authors reported increases in a number of inflammation markers including cytokines (with some variability between the two designs) as well as a statistically significant rise of total IgE antibody levels (Ban et al., 2006).
Arts and colleagues used a "respiratory local lymph node assay", i.e. a study protocol in which male Balb/c mice were first exposed once per day on three consecutive days to HDI or TDI by inhalation, followed by an evaluation of the proliferation of the draining mandibular lymph nodes three days later. Both diisocyanates caused marked proliferation with the stimulation index exceeding a value of 3 at all inhalation concentrations applied (Arts et al., 2008; de Jong et al., 2009).

10.6.5.2.3 Rats

Hoymann and colleagues performed a combined inhalation chronic toxicity and carcinogenicity test in female Wistar rats using MDI. As a result of between 65 and 520 daily 17 h exposures, the author of the summary in the technical dossier noted "*a dose-dependent impairment of the lung function in the sense of an obstructive-restrictive malfunction with diffusion disorder, increased lung weights, an inflammatory reaction with increased appearance of lymphocytes (but not of granulocytes) in the lung in the high dose group as a sign of specific stimulation of the immune system by MDI*" (Hoymann et al., 1995).

10.6.6 Short summary and overall relevance of the provided information on respiratory sensitisation

10.6.6.1 Human data

For TODI, no human data relevant for the classification as a respiratory sensitiser were identified. However, a large database of human data on the source substances HDI, MDI, and TDI provides undeniable proof that these substances are able to cause RS in humans and are therefore rightfully listed as Resp. Sens. 1 in Annex VI to the CLP regulation.

10.6.6.2 Animal data

Again no relevant data for TODI were identified from the available data base. In contrast, exposure to the three source substances by inhalation was shown to trigger RS in a variety of rodent species as demonstrated by the production of specific antibodies, impairment of respiratory function, and characteristic inflammation markers in BALF. Observed respiratory symptoms (increased respiratory rate, effects on respiratory flow, laboured breathing etc.) resemble those seen in humans with asthma.

Skin sensitisation has also been observed following induction via inhalation.

[4] The abstract of this publication claims that induction was performed over „four consecutive days", however, the method section states that induction was performed on „days 0, 1, and 2". Coming from the methods section the latter information is assumed to be more reliable.

Overall, the interdependencies and quantitative contributions to sensitisation of factors such as the species and strain used, concentration and total dose received upon induction, or the temporal pattern of dosing are still poorly understood.

10.6.7 Comparison with the CLP criteria

10.6.7.1 Human data

Section 3.4.2.1.2.3 of Annex I to the CLP regulation states that the evidence required to demonstrate RS in humans *"could be: (a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include: (i) in vivo immunological test (e.g. skin prick test); (ii) in vitro immunological test (e.g. serological analysis); (iii) studies that indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects; (iv) a chemical structure related to substances known to cause respiratory hypersensitivity; (b) data from one or more positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction".* Furthermore, section 3.4.2.1.2.5 notes that "*the results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own"* (European Parliament and Council, 2008).

Since for TODI, no study in humans is available, a category approach is used for classification in accordance with CLP Article 5 1. (2) referring to REACH Annex XI, section 1. Numerous case reports and epidemiological studies with the source substances HDI, MDI, and TDI evaluated for this dossier report positive bronchial provocation tests with these substances and are therefore each sufficient on their own to justify classification for RS. In addition, many of the other criteria mentioned above are met by these reports.

On the other hand, no reliable ERR can be established from the database and therefore no reliable relative or absolute potency estimate can be made. In addition, reading across already unreliable potency information from the three different source substances to the target substance would be associated with a high degree of uncertainty. Moreover, no harmonised approach for sub-categorising respiratory sensitisers is available yet.

Still, these data are sufficient to classify TODI as Resp. Sens. 1 in accordance with the CLP regulation.

10.6.7.2 Animal data

Several studies in guinea pigs, mice, and rats with the source substances HDI, MDI, and TDI were identified in which the production of specific antibodies and the impairment of pulmonary function as a consequence of exposure to diisocyanates via inhalation were demonstrated.

According to the criteria already mentioned above (cf. section 10.6.5: *"data from appropriate animal studies which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans may include: (a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice; (b) specific pulmonary responses in guinea pigs"*), these data lend qualitative support to the observations in humans noted in the previous sub-section.

10.6.8 Conclusion on classification and labelling for respiratory sensitisation

In summary, in a weight-of-evidence decision according to CLP Annex I, section 1.1.1, considering:

- general mechanistic knowledge on the biological effects of diisocyanates,
- a category approach using read-across of human and non-human data from the source substances HDI, MDI, and TDI to the target substance TODI, and
- the potential of TODI to cause skin sensitisation (cf. section 10.7 below),

DE concludes that TODI should be classified as Resp. Sens. 1 (hazard statement H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled) while the available data do not allow for sub-categorisation.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed to classify TODI as Resp. Sens. 1; H334.

There are no specific human or animal data on respiratory sensitisation available for TODI. Therefore, the proposed harmonised classification was based on read across.

Only the three most commonly used diisocyanates were used as source substances, because most of the published literature on diisocyanates relates to these:

- hexamethylene diisocyanate (HDI, CAS number 822-06-0),
- 4,4'-methylenediphenyl diisocyanate (MDI, CAS number 101-68-8) and
- m-tolylidene diisocyanate (TDI, CAS number 26471-62-5; 80/20 mixture of 2,4-TDI and 2,6-TDI isomers).

All three isocyanates have a harmonised classification as Resp. Sens. 1; H334. In addition, the DS noted that several other diisocyanates also have a classification as respiratory sensitiser. For HDI, MDI and TDI, there is an abundance of data available, both human and animal.

Human data for the source substances HDI, MDI and TDI

More than 100 case reports and epidemiological studies were evaluated by the DS. An overview is available in Annex I of the CLH report (tables 2-8). The literature consistently demonstrates the potential of HDI, MDI and TDI to cause respiratory sensitisation in humans. TAll three have harmonised classifications as Resp. Sens. 1; H334.

According to the DS, the case reports provide clear evidence that humans exposed to the source substances may suffer from a broad spectrum of respiratory effects, including asthma and pathological changes of the airways. A number of fatal cases have also been reported, albeit not in recent years. Although during the early stages of the development of the disease respiratory symptoms may eventually be reversed upon removal from exposure, an irreversible remodelling of the airways will eventually take place when exposure is continued. On the other hand, these case reports do not allow for an assessment of the frequency of occurrence of respiratory sensitisation in the human population. They feature only a small number of patients and it is not known which fraction of all exposed individuals is affected and which fraction of those affected is reported. The case reports are therefore not suited for sub-categorisation. In addition, no harmonised approach for sub-categorising respiratory sensitisers is yet available.

According to the DS, despite the large number of available epidemiological studies, none of them are eligible for deriving a reliable Exposure-Response-Relationship (ERR) due to limitations of the studies. This is also inherent in the mechanism of the disease. No study can overcome the challenge of not having sensitive predictive markers for diisocyanate sensitisation. Also, dermal exposure, as well as inhalation peak exposure, likely contribute to the induction of sensitisation but to date it has not been possible to assess this appropriately.

Patients with diisocyanate-induced asthma display both early (seconds to minutes) and delayed (up to several hours) hypersensitivity. However, the prevalence of delayed responses is as high as 70% (Niimi *et al.*, 1996). A particular concern is the delay between onset of (low-

level) exposure at work and the manifestation of the asthmatic symptoms, which may be as long as several years after the start of exposure. In addition, patients often develop persistent bronchial hyperresponsiveness (BHR; often also the more general term "airway hyperresponsiveness/hyperreagibility (AHR)" is used interchangeably) to non-specific stressors including e.g. other chemicals such as methacholine, cold, dust, or physical exercise that can last for years even in the absence of continued exposure, and complete recovery of lung function may never be achieved (Johnson *et al.*, 2004a).

Animal data for the source substances HDI, MDI and TDI

There are no internationally recognised *in vivo* test methods for identification of respiratory sensitisation. Animal studies were considered by the DS to be relevant for the classification only if the induction route was truly via inhalation. Studies using other routes of induction or mixed routes were discarded. Furthermore, studies were considered unreliable and excluded from the assessment in the event that any of the following information was missing or incomplete: identity of the test substance, physical state of the test substance as applied (aerosol or vapor), inhalation protocol followed (whole-body or head/nose-only), confirmation of the presence of a negative control and number of animals per dose group. In addition, the DS noted that animal study designs for respiratory sensitisation (RS) have been manifold, involving a variety of species, protocols and target endpoints. A standardised protocol with regulatory acceptance is still to be developed. Therefore, the DS noted that a negative result from an animal experiment on RS is not sufficient to exclude the need for classification and labelling. Consequently, for the read across assessment, the evaluation concentrated on data providing a positive indication of respiratory sensitisation. Therefore, for HDI, MDI, and TDI, only studies reporting the presence of one or more relevant effects were selected by the DS for further processing. Where several experiments were reported in one study report, only those with effects were processed further.

For HDI, MDI and TDI, 36 experiments from 18 study reports qualified for further evaluation. These are summarised in the Table below. These experiments were performed in guinea pigs (6 with MDI, 14 with TDI), mice (3 with HDI, 7 with TDI) and rats (6 with MDI). The DS concluded that inhalation exposure to the three source substances was shown to trigger respiratory sensitisation, as demonstrated by the production of specific antibodies, impairment of respiratory function and characteristic inflammation markers in bronchoalveolar lavage (BAL) fluid. Observed respiratory symptoms (increased respiratory rate, effects on respiratory flow, laboured breathing etc.) resemble those seen in humans with asthma. In addition, skin sensitisation has also been observed following induction via inhalation. However, the interdependencies and quantitative contributions to sensitisation of factors such as the species and strain used, concentration and total dose received upon induction or the temporal pattern of dosing are still poorly understood.

Table. *Summary by the DS of the animal studies, evaluating the potential of the source substances HDI, MDI, and TDI to cause respiratory sensitisation in rodents following exposure via the inhalation route (sorted by species and year; originally Table 10 in the CLH report).*

Strain	Sex	"Induction" Agent	"Elicitation" Route	"Elicitation" Agent	Physical state	Inhalation type	Animals/group	No. of "induction" exposures	Hours/exposure	Total days	Critical effect	Reference
Guinea pigs												
ESH	F	TDI	-	-	VP	HO	8	2	3	3	AB	(Karol, 1983)
							12	5		5		
			IDE	TDI-GPSA			8				SS	
			INH	TDI-GPSA/ TMI-GPSA			12				RF	
DH	F	TDI	INH	TDI-GPSA	AE	NO	10	5	3	5	AB/RF	(Botham et al., 1988)
DH	F	MDI	-	-	VP	NO	5	5	3	21	AB	(Dearman and Botham 1990)
			IPE	MDI-GPSA						22		
Hartley	F	TDI	INH	TDI	VP	WB	7	5	3	21	AB/IF/RF	(Huang et al., 1993a)
Hartley	F	TDI	INH	TDI	VP	WB	6	5	3	26	AB/RF	(Aoyama et al., 1994)
Hartley	?	MDI	INH	MDI	AE	NO	≥ 8	1	0.25	21/ 22	RF	(Pauluhn, 1994)
				MDI-GPSA								
		TDI		TDI	VP							
				TDI-GPSA								
DH	F	MDI	INH	MDI	AE	NO	16	5	3	18	AB	(Rattray et al., 1994)
?	?	MDI	INH	MDI	AE	NO	16	1	0.25	21/ 28	AB/RF	IUCL: (Bayer, 1995)
DH	F	TDI	-	-	VP	WB	20	1	48	3	RF	(Gagnaire et al., 1996)
									168	8		
DH	F	TDI	-	-	VP	WB	10	1	134 4	56	RF	(Gagnaire et al., 1997)
DH	F	TDI	INH	TDI/TDI-GPSA	VP	NO	8	1	0.25	21	AB/IF/RF	(Pauluhn and Mohr, 1998)
Hartley	F	TDI	TOP	TDI	AE	NO	8	1	4	15	SS	(Ebino et al., 2001)

Strain	Sex	"Induction" Agent	"Elicitation" Route	"Elicitation" Agent	Physical state	Inhalation type	Animals/group	No. of "induction" exposures	Hours/exposure	Total days	Critical effect	Reference
Mice												
C57BL/6	F	TDI	INH	TDI	VP	NO	5	30	4	56	AB/IF/RF	(Matheson et al., 2005a
C57BL/6	F	TDI	INH	TDI	VP	HO	5	1	2	1	AB/IF/RF	(Matheson et al., 2005b
								30	4	56		
BALB/c	F	TDI	INH	TDI	VP	WB	6-8	1	4	14	AB/IF	(Ban et al., 2006)
BALB/c	M	HDI	-	-	VP	NO	6	3	0.75	5	IF	(Arts et al., 2008; de Jong et al., 2009)
									1.5			
									3			
		TDI							0.75			
									1.5			
									3			
Rats												
Wistar	F	MDI	-	-	AE	WB	8	436	17	610	RF	IUCL: (Hoymann et al. 1995)
							12					
							20	65		98	IF	
								260		365		
								436		371		
							80	520		728		

AB=antibodies; AE=aerosol; DH=Dunkin-Hartley; ESH=English smooth-hair; HO=head-only; IDE=intradermal; IF=inflammation; INH=inhalation; IPE=intraperitoneal; NO=nose-only; RF=respiratory function; SS=skin sensitisation; TOP=topical; WB=whole-body; VP=vapour

Read across from HDI, MDI and TDI to TODI

The read across of data was based on the category approach and structural similarity to monomeric diisocyanates, according to the ECHA Read Across Assessment Framework (RAAF) Scenario 6 (human health). In this scenario, the read across hypothesis is based on different compounds that have qualitatively similar properties, with no relevant variations in properties observed among source substances and the same strength predicted for the target substance. All assessment elements (AEs) relevant to the RAAF Scenario 6 (human health) were considered by the DS.

The three source substances and the target substance TODI all share the structural feature of two isocyanate functional groups, while the part of the molecular structure that links the two isocyanate groups are variable (Figure below).

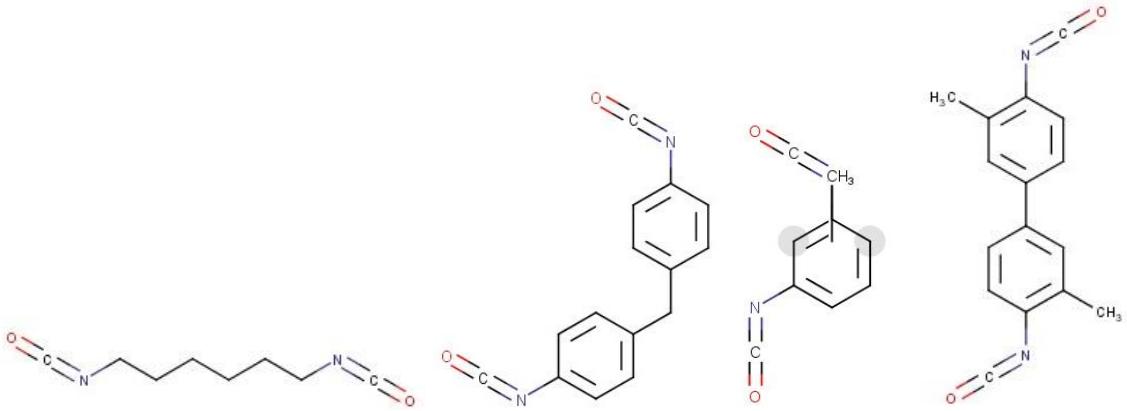


Figure. The structures of HDI, MDI, TDI and TODI, respectively, from left to right.

The isocyanate functional group is a well-known structural alert for respiratory sensitisation and is therefore commonly used also in respiratory sensitisation prediction tools. It has been hypothesised, and to a certain degree demonstrated, that similarly to skin sensitisation, covalent binding of electrophiles to proteins in the lung triggers the molecular initiating event (MIE) of the sensitisation mechanism. In the case of isocyanates, an acylation type reaction between electrophilic N=C=O functional groups and nucleophilic protein moieties may occur leading to the formation of protein adducts (Enoch *et al.*, 2011; Enoch *et al.*, 2009; Enoch *et al.*, 2014). Furthermore, it has been noted that a higher occupational asthma hazard is caused by low molecular weight agents that can form two or more bonds with human macromolecules, and that e.g. diisocyanates rank highly in this respect (Agius *et al.*, 2000). The potential reactivity of HDI, MDI and TDI towards amino acids has been shown *in chemico* (Lalko *et al.*, 2013).

Moreover, the DS noted that at least the qualitative respiratory sensitising potential of HDI, MDI and TDI appears to be dependent on the diisocyanate structure. The variations in the molecular structure connecting the two groups are of less importance. However, they may have an impact on the physico-chemical and ADME properties of the compounds and therefore influence their relative potencies, although this was not addressed in the dossier.

Comments received during consultation

One MSCA commented on the proposed classification for respiratory sensitisation and supported Resp. Sens. 1; H334. In addition, Industry also commented and agreed with the proposed classification.

Assessment and comparison with the classification criteria

There are no validated test methods for respiratory sensitisation and therefore compounds are typically classified based on human data, with supportive evidence from e. g. animal data. Furthermore, there is no specific human or animal data available for TODI that could be used to assess respiratory sensitisation. However, animal data on skin sensitisation (discussed below) demonstrates that TODI has sensitising properties

For the source substances HDI, MDI and TDI, numerous case reports and epidemiological studies consistently demonstrate potential to cause respiratory sensitisation in humans. *In vivo* studies provide additional support. Consequently, all three source substances have existing harmonised classification as Resp. Sens. 1; H334, as do also many other diisocyanates. Current mechanistic knowledge on the mode of action of diisocyanates indicates that the effects depend exclusively on the diisocyanate group, while the remaining of the molecule can vary considerably. In other words, it is the diisocyanate structure itself that is widely considered to be an alert for respiratory sensitisation.

For TODI, the read across performed by the DS considered all of the AEs relevant for scenario 6 of the RAAF (see RAAF Appendix F).

In addition to the classification criteria, the CLP Regulation, Annex I, section 3.4.2.1.2.3 states that the evidence required to demonstrate respiratory sensitisation in humans *"could be: (a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include: (i) in vivo immunological test (e.g. skin prick test); (ii) in vitro immunological test (e.g. serological analysis); (iii) studies that indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects; (iv) a chemical structure related to substances known to cause respiratory hypersensitivity; (b) data from one or more positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction".* Furthermore, section 3.4.2.1.2.5 notes that *"the results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own"*.

Regarding *in vivo* studies, section 3.4.2.1.3.1 of the same Annex states: *"data from appropriate animal studies which may be indicative of the potential of a substance to cause sensitisation by inhalation in humans may include: (a) measurements of Immunoglobulin E (IgE) and other specific immunological parameters in mice; (b) specific pulmonary responses in guinea pigs".*

As no studies in humans or animals are available for TODI, category-based read-across is used for classification, in accordance with CLP Article 5(1)(c), which in turn refers to the methods listed in section 1 of REACH (EC 1907/2006) Annex XI.

Overall, RAC considers the WoE assessment by the DS to be adequate. In addition, RAC agrees with the justification for a category approach using read across (based on human and non-human data) from the Cat. 1 respiratory sensitisers HDI, MDI and TDI to the target substance TODI. The read across by the DS is acceptable and has been performed according to the RAAF. RAC also agrees that it is not possible to assign TODI into sub-categories 1A or 1B, as no reliable data on the potency of either TODI or the source substances HDI, MDI or TDI are available.

In conclusion, RAC agrees with the DS that **classification as Resp Sens. 1, H334 is warranted** for TODI. Although HDI, MDI and TDI all have SCLs (C ≥ 0.5%, 0.1% and 0.1%, respectively), no SCL was proposed by the DS for TODI. RAC is of the opinion that in the absence of specific data for TODI, it is not possible to determine an SCL.

10.7 Skin sensitisation

To the knowledge of DE, no studies of the skin sensitising potential of TODI in humans are available. However, skin sensitisation test data in animals summarised in Table 9 below are available which are sufficient for classification and labelling. Therefore, in this case read-across from other diisocyanates is not necessary. Nevertheless it is stressed that all diisocyanates currently classified as respiratory sensitisers in Annex VI to the CLP regulation also are classified as skin sensitisers or, in the case of naphthylene diisocyanate (NDI, CAS 3173-72-6) have data showing their skin sensitisation potential.

Table 9: Summary table of the available animal studies on skin sensitisation for TODI

Method, guideline, deviations	**Species, strain, sex, no/group**	**Test substance, vehicle**	**Study protocol**	**Results**	**Reference**
OECD TG 406 (GPMT)/EU B.6 Reliability 2 (reliable with restrictions): Only summary available	Guinea pig, Dunkin-Hartley, female, 10/test group, 5/control	TODI, Arachis oil BP/acetone	Induction *Intradermal (Day 0)* Three pairs of injections: ▪ Freund's Complete Adjuvant (FCA)/ distilled water 1:1, ▪ 0.1% w/v formulation of the test material in arachis oil BP, ▪ 0.1% w/v formulation of the test material in a 1:1 preparation of FCA plus distilled water. *Topical (Day 7)* 50 % w/w TODI in acetone, 48 h, occlusive Challenge (Day 21) Topical, 50% and 25 % w/w TODI in acetone, 24 h, occlusive	80-90% sensitisation rate at both challenge doses of 50 and 25% at all observation time points (24, 48, and 72 h post-challenge) For details, cf. Table 10 Extreme skin sensitiser; Skin Sens. 1A	(Safepharm, 1998)

Table 10: Results obtained in the GPMT test with TODI (Safepharm, 1998)

Reading/hours post-challenge	**Group**	**Conc.**	**No. with reactions/ total no. in group (%)**	**Remarks on result**
1st/24	Test	25%	8/10	Positive indication of skin sensitisation
	Neg. control	25%	0/5	No indication of skin sensitisation
	Test	50%	9/10	Positive indication of skin sensitisation
	Neg. control	50%	0/5	No indication of skin sensitisation
2nd /48	Test	25%	9/10	Positive indication of skin sensitisation
	Neg. control	25%	0/5	No indication of skin sensitisation
	Test	50%	8/10	Positive indication of skin sensitisation
	Neg. control	50%	0/5	No indication of skin sensitisation
3rd/72	Test	25%	9/10	Positive indication of skin sensitisation
	Neg. control	25%	0/5	No indication of skin sensitisation
	Test	50%	9/10	Positive indication of skin sensitisation
	Neg. control	50%	0/5	No indication of skin sensitisation

In a guinea pig maximisation test (GPMT), TODI produced a 80-90% (8-9/10) sensitisation rate at all challenge concentrations and observation time-points. It was concluded that under the conditions of this assay, TODI was a potent skin sensitiser. For a detailed summary of this study, the reader is referred to Annex I to this dossier (Safepharm, 1998).

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

While no relevant human data on skin sensitisation caused by TODI were identified, the available GPMT demonstrates the potential of TODI to act as a skin sensitiser with extreme potency in guinea pigs.

10.7.2 Comparison with the CLP criteria

According to the criteria given in Table 3.4.3 of the CLP regulation, skin sensitisers fall into Skin Sens. sub-category 1A based on the results from a GPMT test, if 30% or more of the animals show a positive response at an intradermal induction concentration of ≤ 0.1%. This criterion is fulfilled for the available GPMT in which at all observation time-points 80-90% of the treated animals showed a positive sensitisation reaction with an intradermal induction concentration of 0.1%. Moreover, according to Table 3.7 of the CLP guidance with a 80-90% sensitisation rate at an intradermal induction concentration of 0.1%, TODI qualifies as an "Extreme Sensitiser" for which the setting of a Specific Concentration Limit (SCL) of 0.001% is recommended in Table 3.9 (ECHA, 2017a).

Table 11: Comparison of experimental results confirming the skin sensitisation potential with TODI in animals with the respective criteria of the CLP regulation and the CLP guidance

Criteria acc. to Table 3.4.3 and Table 3.4.4 of the CLP regulation and Table 3.7 of the CLP guidance		**Reference(s)**	**Sensitisation rate (%)/Intradermal induction dose (%)**	**Resulting Classification**
GPMT				
Skin Sens. 1A, Extreme	≥ 60% responding at ≤ 0.1% intradermal induction dose	(Safepharm, 1998)	80-90/0.1	**Skin Sens. 1A** **Extreme sensitiser** **SCL 0.001% (w/w)**
Skin Sens. 1A, Strong	30% to < 60% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0.1 to 1% intradermal induction dose			
Skin Sens. 1B, Moderate	30% to < 60% responding at > 0.1% to 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose			

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the test results in guinea pigs, TODI should be classified as Skin Sens. 1A (hazard statement H317: May cause an allergic skin reaction) and an SCL of 0.001% should be assigned in line with the recommendations in Table 3.9 of the CLP guidance (ECHA, 2017a).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No information on the skin sensitising potential of TODI in humans is available.
One animal study is available (Safepharm, 1998) but only the summary from the lead registrant was available. It is a Guinea pig maximisation test (GPMT), performed according to OECD TG 406 and it is stated to have been conducted under GLP.

In 10 female Dunkin-Hartley Guinea pigs per group (5 animals in the negative control group), TODI (99.9% purity) produced a 80-90% (8-9/10) sensitisation rate at both challenge concentrations and at all observation time-points (24h, 48h and 72h post-challenge) following intradermal induction with 0.1% w/v formulation of the test material in arachis oil BP, topical induction with 50% w/w TODI in acetone and topical challenge with 50% and 25 % w/w TODI in acetone.
There was no indication of skin sensitisation in negative controls and no effect on body weight gain in any group. A dose range finding test was performed before the main study. A positive control was not included. The results are summarised in the Table below

***Table**. GPMT study results (Table 10 from Annex 1 to the CLH report)*

Reading/hours post-challenge	Group	Conc.	No. with reactions/ total no. in group (%)	Clinical observations	Remarks on result
1st/24	Test	25%	8/10	See section „Results"	Positive indication of skin sensitisation
	Neg. control	25%	0/5	None	No indication of skin sensitisation
	Test	50%	9/10	See section „Results"	Positive indication of skin sensitisation
	Neg. control	50%	0/5	None	No indication of skin sensitisation
2nd /48	Test	25%	9/10	See section „Results"	Positive indication of skin sensitisation
	Neg. control	25%	0/5	None	No indication of skin sensitisation
	Test	50%	8/10	See section „Results"	Positive indication of skin sensitisation
	Neg. control	50%	0/5	None	No indication of skin sensitisation
3rd/72	Test	25%	9/10	See section „Results"	Positive indication of skin sensitisation
	Neg. control	25%	0/5	None	No indication of skin sensitisation
	Test	50%	9/10	See section „Results"	Positive indication of skin sensitisation
	Neg. control	50%	0/5	None	No indication of skin sensitisation

The Dossier Submitter concluded that these results warrant classification in Skin Sens. sub-category 1A, according to the criteria given in Table 3.4.3 of the CLP regulation (30% or more of the animals show a positive response at an intradermal induction concentration of $\leqslant$0.1%).

Also, a Specific Concentration Limit (SCL) of 0.001% has been proposed (according to Table 3.9 in ECHA Guidance (ECHA, 2017a)), since according to Table 3.7 of the CLP guidance, a 80-90% sensitisation rate at an intradermal induction concentration of 0.1%, qualifies TODI as an "Extreme Sensitiser".

Comments received during consultation

Two comments (one from industry and one from an MSCA) were received, supporting the proposed classification (Skin Sens. sub-category 1A) and the SCL (0.001%).

Assessment and comparison with the classification criteria

RAC considers that for regulatory purposes, the summary of the Safepharm (1998), study performed under GLP in accordance with OECD TG 406 (GPMT guideline), provides sufficient information on study methodology and results, despite some limitations (skin readings were impeded by residual test material and incidents of physical damage caused by attempted removal of adhered test material; no positive control).

According to the criteria defined in the CLP Regulation Skin Sens. sub-category 1A is applicable when there are ≥30% responding animals at ≤0.1% intradermal induction dose in a GPMT. RAC, therefore, agrees with the Dossier Submitter that the results of this study justify **classification of TODI as Skin Sens. sub-category 1A**, since 80-90% of tested animals had a positive reaction following 0.1% intradermal induction dose.

According to ECHA CLP Guidance (Table 3.7) this magnitude of response indicates that it is a skin sensitiser with extreme potency. Therefore, an **SCL of 0.001%**, as proposed by the Dossier Submitter, is considered warranted (ECHA CLP Guidance, Table 3.9).

10.8 Germ cell mutagenicity

10.8.1 Evaluation strategy

Some *in vitro* and *in vivo* studies are available to evaluate TODI mutagenicity. However, other data exists on similar substances which can be used to bring useful information for the evaluation of mutagenicity potential of TODI.

Concerning carcinogenicity, no data on TODI is available. The only repeated study available is a 28-day study by oral route which is too short to highlight carcinogenic potential (Anonymous, 1998b).

Therefore, based only on data from TODI, no robust assessment of mutagenic and carcinogenic potential is possible.

Consequently, an evaluation strategy using read-across of human and non-human data from structurally similar substances to the target substance has been performed to assess the potential of TODI to cause germ cell mutagenicity and carcinogenicity. This is described below.

MDI and TDI, commonly used diisocyanates, are classified as Carc. 2 according to CLP Regulation. These substances form MDA and TDA by hydrolysis (in a similar way as TODA is formed from TODI). MDA and TDA have an harmonized classification as Carc. 1B and Muta. 2 and TODA has an harmonized classification as Carc. 1B. These data suggest that TODI needs to be assessed for the classification of these endpoints.

The approach used by DE for respiratory sensitisation (10.6.2) cannot be used mainly due to the lack of a known mechanism of action linked to the isocyanate group. Moreover, considering the existing harmonised classification of the metabolites substances TODA, MDA and TDA, the DS extends the list of substances included in the evaluation.

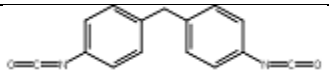
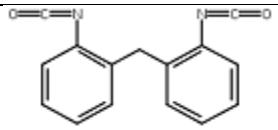
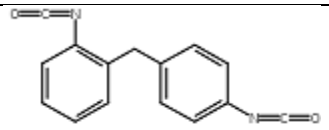
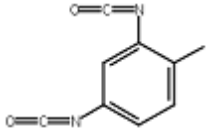
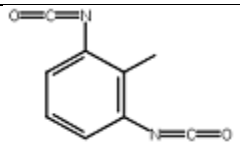
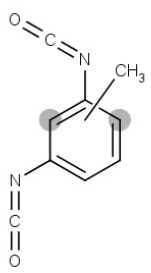
Considering therefore that:

- **no robust dataset is available on mutagenicity of TODI;**
- rapid and complete hydrolysis of isocyanate substances is expected;
- **data on mutagenicity and carcinogenicity are available for other substances which belong to the diisocyanates group and their hydrolysis products;**
- **a mechanism of carcinogenicity for MDI is proposed (increase regenerative proliferation of type-II cells is considered to be the cause of the pre-neoplastic changes in rats, which is a known chronic reaction of rat lung to irritating substances),**

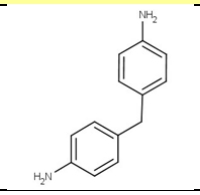
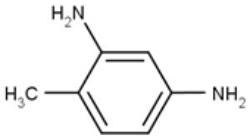
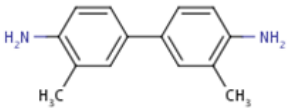
an assessment of the mutagenicity and carcinogenicity of TODI in a weight of evidence approach seems adequate.

Table 612 provides information on the identity and harmonised classification of the source substances.

Table 12: Diisocyanates and metabolites used for evaluation strategy with a harmonised classification (excluding polymers).

EC Name	Abbreviation	EC No.	CAS No.	Structure	Classification
methylenediphenyl diisocyanate	MDI (group)	247-714-0	26447-40-5		Carc 2 H351
4,4'-methylenediphenyl diisocyanate	4,4'-MDI	202-966-0	101-68-8		Carc 2 H351
2,2'-methylenediphenyl diisocyanate	2,2'-MDI	219-799-4	2536-05-2		Carc 2 H351
o-(pisocyanatobenzyl)phenyl isocyanate	2,4'-MDI	227-534-9	5873-54-1		Carc 2 H351
4-methyl-m-phenylene diisocyanate	2,4-TDI	209-544-5	584-84-9		Carc 2 H351
2-methyl-m-phenylene diisocyanate	2,6-TDI	202-039-0	91-08-7		Carc 2 H351
m-tolylidene diisocyanate	80/20 TDI or 65/35 TDI	247-722-4	26471-62-5		Carc 2 H351

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3,3'-DIMETHYLBIPHENYL-4,4'-DIYL DIISOCYANATE

EC Name	Abbreviation	EC No.	CAS No.	Structure	Classification
4,4'-methylenedianiline	MDA	202-974-4	101-77-9		Carc 1B H350 Muta 2 H341
4-methyl-m-phenylenediamine	TDA	202-453-1	95-80-7		Carc 1B H350 Muta 2 H341
4,4'-bi-o-toluidine	TODA	204-358-0	119-93-7		Carc 1B H350

As described in the Table 614, all members of the group are monomeric diisocyanates, i.e. they share the structural feature of two isocyanate functional groups, or monomeric diamines, which are the hydrolysis products of the former.

Unfortunately, the classifications of these substances are old, and ground for these classifications cannot be found. However, TDI and MDI were assessed by IARC in monograph volume 71 (1999) and TODA in monograph 1 (1987). TDI isomers are possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals. MDI (industrial preparation) is not classifiable as to its carcinogenicity to humans (Group 3) based on inadequate evidence in humans and limited evidence in experimental animals. TODA was classified possibly carcinogenic to humans (Group 2B) based on no adequate data in humans and sufficient evidence in experimental animals.

Three *in vitro* genetic toxicity studies with TODI (Ames test, Gene cell mutation and chromosomic aberration, with and without simulated metabolic activation) are available, detecting mutations and clastogenic potential (Table 13).

Table 13: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD test guideline 471 (bacterial reverse mutation assay)	TODI with a purity >99.9% The vehicle was DMSO.	The test strains S. typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100, TA 102, TA 104 and E. coli WP2 uvr A as well as E. coli WP2 uvr A pKM 101 were examined at 10, 20, 50, 100, 200, 500, 1000, 2000 µg TODI/plate with and without metabolic activation.	Positive results were obtained in the presence of metabolic activation for TA 98 and TA 1538 at concentrations of 10 to 1000 µg/plate (an evaluation of 2000 µg/plate was not possible due to growth inhibition).	Anonymous / JETOC (1996)
OECD test guideline 476 (In vitro	TODI with a purity >99.9% The vehicle used	Mouse lymphoma L5178Y cells were exposed to the tested material in 3 independent experiments, at the following	TODI induced small but statistically significant increases in mutant frequency in each of 3 independent experiments (without metabolic activation in experiment 1 (dose-related), with	Anonymous (1999a)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
gene mutation test in mammalian cells)	was acetone.	concentrations: - experiment 1: 2, 4, 8, 12, 16 µg/mL with and without metabolic activation (3h exposure); - experiment 2: 4, 8, 16, 20, 24 µg/mL without metabolic activation (24h exposure) and 4, 8, 12, 14, 16 µg/mL with metabolic activation (3h exposure); - experiment 3: 4, 6, 8, 10, 12 µg/mL without metabolic activation and 6, 8, 10, 12, 14 µg/mL with metabolic activation (3h exposure)	metabolic activation in experiment 2 (dose-related), and with (dose-related) and without metabolic activation in experiment 3).	
Similar to OECD test guideline 473	TODI with a purity of 99.8%. The vehicle was DMSO.	CHL cells were exposed to the test material at the following concentrations: 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL (24h, 48h, without metabolic activation) and 0.2, 0.3, 0.4, 0.5, 0.6 mg/mL (6h, with and without metabolic activation). The vehicle was DMSO.	Slightly positive results were obtained with metabolic activation at 0.6 mg/mL.	Anonymous / JETOC (1996)

Moreover, as described in Table 14, two different *in vivo* genetic toxicity studies with TODI detecting mutations and aneugenic activity (UDS and micronuclei) are available.

Table 14: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD test Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	TODI with a purity > 99.9%. The vehicle was arachis oil.	Albino Crl:CD-1TM (ICR) BR mice (males/females) were exposed by intraperitoneal administration to the test substance in a single dose at the nominal concentrations of 125 mg/kg bw (sacrifice 24h after exposure), 250 mg/kg bw (sacrifice 24h after exposure) and 500 mg/kg bw (sacrifice 24h and 48h after exposure) following a range-finding	No significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice was observed under the conditions of the test. The test was considered negative.	Anonymous (1998a)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		assay.		
GLP-compliant unscheduled DNA synthesis (DNA damage and/or repair) conducted in accordance with OECD test Guideline 486 (Test with Mammalian Liver Cells in vivo)	TODI with a purity of 99.8% (range 99.5-100%). The vehicle was arachis oil.	Crj: CD(SD) rats (males) were exposed by gavage to the test material at the nominal concentrations of 700 and 2000 mg/kg bw (experiment 1: perfusion 16h after dosing; experiment 2: perfusion 2h after dosing), following a range-finding assay.	No signs of toxicity were observed. No increase in the incidence of unscheduled DNA synthesis was observed at any time point. The test was considered negative.	Anonymous (1999b)

10.8.2 Short summary and overall relevance of the provided information on germ cell mutagenicity

TODI was tested in three *in vitro* genetic toxicity studies (Ames test, Gene cell mutation and chromosomic aberration, with and without simulated metabolic activation) and two different *in vivo* genetic toxicity studies (UDS and micronuclei).

In these assays, the tested substance TODI has a high purity (typical purity 99.8% with a range of 99.5%-100%).

In the Ames test on 6 bacterial strains, positive results were obtained in the presence of metabolic activation for two strains (TA 98 and TA 1538 at concentrations of 10 to 1000 µg/plate).

In an *in vitro* gene mutation test in mammalian cells, TODI induced small but statistically significant increases in mutant frequency in each of 3 independent experiments (without metabolic activation in experiment 1 (dose-related), with metabolic activation in experiment 2 (dose-related), and with (dose-related) and without metabolic activation in experiment 3).

A Chromosome Aberration Test in CHL cells was performed to assess the mutagenicity potency of TODI. The reported data of this Chromosome Aberration Test shows, that under the experimental conditions described, TODI induced chromosome aberration after metabolic activation at 0.6 mg/mL.

TODI is unstable in water and, therefore, DMSO and acetone were used in *in vitro* tests and arachis oil in *in vivo* tests. The impact of the vehicle on the test results was however not studied such as the stability of TODI in organic solvents and the identity of relevant degradation products.

Diisocyanates were shown to be unstable in aprotic polar solvents such as dimethylsulfoxide (DMSO), resulting in the formation of amines. For assessing the in vitro genotoxicity of TODI, DMSO and acetone (also an aprotic polar solvent) were used. Based on the available information on structurally similar aromatic diisocyanates, degradation of TODI into TODA (4,4'-bi-o-toluidine, CAS 119-93-7, EC 204-358-0) in aprotic polar solvents cannot be excluded, and it is not possible to conclude whether the positive results observed in the *in vitro* tests are due to TODI and/or TODA and/or other degradation products. TODA is not registered under REACH and therefore no registration dossier is available; however TODA has a harmonised classification as Carc. 1B. Even if there is no harmonised classification for mutagenicity, data found in the literature for TODA are equivocal (NTP report n°390; You et al. (1993); HSDB data bank; IARC monography

on benzidine and derivatives (2010)). Therefore, no clear conclusion on genotoxicity mechanism can be drawn based on these *in vitro* tests related to TODA.

Concerning *in vivo* tests, TODI will likely react with the vehicle (arachis oil) to form a long fatty chain with TODA in one extremis. This may affect the results of the micronucleus study to an unknown extent (considering that intraperitoneal administration was used), and which will likely affect the results of the UDS study by preventing gastro-intestinal absorption and distribution of the substance to the target tissues. In a 28-day study with administration of TODI by oral route in arachis oil, absorption of the test material seems poor as residual material was found in the gastrointestinal tract (Anonymous, 1998).

In conclusion, UDS test is unsuitable to conclude on the mutagenicity concern, considering that no data is available to support that TODI has been absorbed in the gastro-intestinal tract and has been able to reach the target tissues. Moreover, this method is not considered suitable to assess genotoxic carcinogens as it is considered of low sensitivity.

Even if data are available on TODI, mutagenicity data of structurally similar MDI (a diisocyanate) can be addressed. It appears that most of the available test results of *in vitro* genotoxicity assays for 4,4'-MDI rather reflect the properties of reaction products formed under specific assay conditions than the ones of the parent compound.

A key study was performed in accordance with the OECD 489 to assess the potential of aerosolized 4,4'-MDI to cause DNA damage to the lung and liver of male Wistar rats following a single, 6-hour nose-only inhalation exposure to the concentrations of 2, 5, and 11 mg/m^3 (achieved 2.5, 4.9, and 12 mg/m^3). The tested substance did not cause a significant increase in DNA damage in the lung (as evaluated in cells obtained from bronchoalveolar lavage, BAL cells), liver, and stomach under the test conditions. Therefore, 4,4'-MDI was concluded to be negative for the *in vivo* Comet assay under the test conditions.

It can be concluded that under the key study test conditions MDI did not show genotoxic potential, therefore the concern for genotoxic mode of action was not confirmed for this substance (Anonymous, 2016).

10.8.3 Comparison with the CLP criteria

Toxicological results	**CLP criteria**
No human data is available. Thus, a classification category 1A is not appropriate for TODI.	The classification in Category 1A is based on positive evidence from human epidemiological studies.

Testing *in vitro*: Bacterial reverse mutation assays: Positive Tests involving mammalian cells: - Positive (*In vitro* Mammalian Cell Gene Mutation Test) - Positive (*In Vitro* Mammalian Chromosomal Aberration Test) Testing *in vivo* (experiments in mammals): - Negative (Mammalian Erythrocyte Micronucleus Test) - Negative (UDS test) In conclusion, no positive *in vivo* study are available for TODI. In a grouping approach with other diisocyanates such as structurally similar MDI. It appears that most of the available test results of *in vitro* genotoxicity assays for 4,4'-MDI rather reflect the properties of reaction products formed under specific assay conditions than the ones of the parent compound. MDI was not classified as genotoxic by Estonia in 2018. Then, TODI cannot be classified as a Germ cell mutagen according to CLP Regulation.	The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens. If there are positive in vitro data from mammalian mutagenicity assays, structural similarities not sufficient for grouping/read-across may still warrant classification.

10.8.4 Conclusion on classification and labelling for germ cell mutagenicity

In conclusion, the available data do not allow classifying TODI for mutagenicity.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Genotoxicity of TODI has been evaluated in three *in vitro* genotoxicity studies, in one *in vivo* bone marrow micronucleus assay and one *in vivo* liver unscheduled DNA repair study. These have been summarized in the table below. Although *in vitro* studies showed some positive results, the *in vivo* studies remained negative. The DS noted that the stability of TODI in the vehicles used was not investigated. TODI, like other similar diisocyanates, is unstable in water and in aprotic polar solvents, resulting in the formation of amines. For assessing the *in vitro* genotoxicity of TODI, aprotic solvents DMSO and acetone were used, which may result in the degradation of TODI into TODA (4,4'-bi-o-toluidine, CAS 119-93-7, EC 204-358-0) and it is not possible to conclude whether the positive results observed

in the *in vitro* tests are due to TODI and/or TODA and/or other degradation products. Although concerns were expressed relating to the stability and ability of TODI to reach the target tissue in *in vivo* studies, since there is no positive data from *in vivo* studies, no classification for mutagenicity was proposed.

***Table**. Summary table of mutagenicity/genotoxicity tests in vitro and in vivo (Tables 13 and 14 from the CLH report)*

Method	Test substance	Study conditions	Results	Reference
OECD test guideline 471 (bacterial reverse mutation assay)	TODI with a purity >99.9% The vehicle was DMSO.	The test strains *S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98, TA 100, TA 102, TA 104 and *E. coli* WP2 uvr A as well as *E. coli WP2 uvr A pKM 101* were examined at 10, 20, 50, 100, 200, 500, 1000, 2000 µg TODI/ plate with and without metabolic activation.	Positive results were obtained in the presence of metabolic activation for TA 98 and TA 1538 at concentrations of 10 to 1000 µg/plate (an evaluation of 2000 µg/plate was not possible due to growth inhibition).	Anonymous / JETOC (1996)
OECD test guideline 476 (In vitro gene mutation test in mammalian cells)	TODI with a purity >99.9% The vehicle used was acetone.	Mouse lymphoma L5178Y cells were exposed to the tested material in 3 independent experiments: Experiment 1: 2, 4, 8, 12, 16 µg/mL with and without metabolic activation (3h exposure); Experiment 2: 4, 8, 16, 20, 24 µg/mL without metabolic activation (24h exposure) and 4, 8, 12, 14, 16 µg/mL with metabolic activation (3h exposure); Experiment 3: 4, 6, 8, 10, 12 µg/mL without	TODI induced small but statistically significant increases in mutant frequency in each of 3 independent experiments (without metabolic activation in experiment 1 (dose-related), with metabolic activation in experiment 2 (dose-related), and with (dose-related) and without metabolic	Anonymous (1999a)

		metabolic activation and 6, 8, 10, 12, 14 µg/mL with metabolic activation (3h exposure)	activation in experiment 3).	
Similar to OECD test guideline 473	TODI with a purity of 99.8%. The vehicle was DMSO.	CHL cells were exposed to the test material at the following concentrations: 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL (24h, 48h, without metabolic activation) and 0.2, 0.3, 0.4, 0.5, 0.6 mg/mL (6h, with and without metabolic activation). The vehicle was DMSO.	Slightly positive results were obtained with metabolic activation at 0.6 mg/mL.	Anonymous / JETOC (1996)
OECD test Guideline 474 (Mammalian Erythrocyte Micronucleus test)	TODI with a purity > 99.9%. The vehicle was arachis oil	Albino Crl:CD-1TM (ICR) BR mice (males/females) were exposed by intraperitoneal administration to the test substance in a single dose at the nominal concentrations of 125 mg/kg bw (sacrifice 24h after exposure), 250 mg/kg bw (sacrifice 24h after exposure) and 500 mg/kg bw (sacrifice 24h and 48h after exposure) following a range-finding assay.	No significant increase in the frequency of micronuclei in polychromatic erythrocytes of mice was observed under the conditions of the test. The test was considered negative.	Anonymous (1998a)
GLP-compliant unscheduled DNA	TODI with a purity of 99.8% (range 99.5-100%).	Crj: CD(SD) rats (males) were exposed by gavage to the test material at the	No signs of toxicity were observed. No increase in the	Anonymous (1999b)

synthesis (DNA damage and/or repair) conducted in accordance with OECD test Guideline 486 (Test with Mammalian Liver Cells *in vivo*)	The vehicle was arachis oil.	nominal concentrations of 700 and 2000 mg/kg bw (Experiment 1: perfusion 16h after dosing; Experiment 2: perfusion 2h after dosing), following a range-finding assay.	incidence of unscheduled DNA synthesis was observed at any time point. The test was considered negative.	

Comments received during consultation

Comments were received from 2 MSCA and one company and all supported no classification for mutagenicity.

Assessment and comparison with the classification criteria

An OECD TG 471 compliant bacterial reverse mutation test with TODI resulted in positive responses in the presence of metabolic activation in two of the tested strains. An *in vitro* gene mutation test in mammalian cells resulted in small but statistically significant increases in mutant frequency in three independent experiments. In *in vitro* chromosome aberration test, slightly positive results were observed at the highest dose after 6 h exposure, but not after 24 or 48 h exposure with metabolic activation. Since TODI may form the respective amine TODA in water and in aprotic polar solvents, it cannot be determined whether the slight positive responses observed in these *in vitro* studies were due to TODI or to TODA formed under the test conditions.

In vivo, negative results were obtained in a bone marrow micronucleus assay in mice after intraperitoneal (ip) administration of TODI. Also, a test for unscheduled DNA synthesis in liver after gavage administration in rats was negative. The overall picture resembles the data on the structurally similar diisocyanate, MDI. With MDI *in vitro* positive responses have also been obtained, but it is uncertain whether these reflect the properties of reaction products formed under specific assay conditions more than the properties of the parent compound. No clear evidence on *in vivo* genotoxicity of MDI exists. Therefore, MDI has not been classified for mutagenicity.

Comparison to classification criteria

Since no human data is available on TODI, classification in category 1A is not appropriate.

Classification in category 1B requires either positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has

potential to cause mutations to germ cells. Since the available *in vivo* studies for TODI remained negative, category 1B does not apply.

Classification in category 2 also requires positive data from somatic cell mutagenicity/genotoxicity tests *in vivo*. However, substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show a chemical structure-activity relationship to known germ cell mutagens, can be considered for classification as Category 2 mutagens. In the case of TODI, similar diisocyanates like MDI, have not been classified as mutagens because of the lack of clear positive responses in mutagenicity/genotoxicity tests *in vivo*.

In conclusion, **no classification of TODI for mutagenicity is proposed.**

10.9 Carcinogenicity

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Data available in the literature on the carcinogenic effects of these substances in animals are presented below. There is inadequate evidence of carcinogenicity in humans.

For **MDI**, tumours in the lungs were observed in rodents in a chronic toxicity/carcinogenicity inhalation study. No carcinogenicity studies by oral or dermal route are available.

A reliable 2-year chronic toxicity/carcinogenicity inhalation study in rats Wistar with pMDI (1990) is available where formation of a pulmonary adenocarcinoma in one male as well as pulmonary adenomas, described as rare in this strain, in males (6/60) and females (2/59) exposed to 6.03 mg/m^3 of pMDI was found. A non-genotoxic mode of action for tumours formation was claimed by the Registrant(s) due to observation of chronic inflammation/irritation in the lungs following lifetime inhalation exposure.

The evidence of increased lung tumour formation in rats following lifetime inhalation of MDI is described in expert review article (Feron et al., 2001). A non-genotoxic mechanism of MDI action in the lung is indicated. However, epidemiological data does not indicate an increased risk of cancer for workers exposed to MDI.

Feron et al. (2001) performed a comparison of the pulmonary effects described in female rats after chronic inhalation exposure to either polymeric or monomeric MDI (Reuzel et al., 1994 and a chronic inhalation study, 1995). The major pulmonary effects observed included interstitial fibrosis, hyperplasia and bronchiolo-alveolar adenomas, the latter occurring at low incidence in the high exposure groups of both studies (i.e. total inhalation exposures of 17728 and 17575 mg MDI h/m³). Both studies also report the presence of particle-laden macrophages, predominantly in the alveoli close to the alveolar ducts which in some cases, particularly in high dose groups, were associated with areas of fibrosis. It was concluded that the results of the two studies could be combined to serve as a basis for human risk assessment of MDI.

Once deposited in the bronchioloalveolar region of the lung, MDI particles interact chemically with protein and other biological macromolecules reducing their concentrations in the lining surface of the lung. To maintain normal homeostasis, increased synthesis of secretory proteins by Type II pneumocytes is induced. As the increased synthesis becomes maximized but demand for protective proteins is maintained there is a secondary, compensatory response characterized by an increase in cell replication, resulting in bronchioloalveolar hyperplasia in the terminal bronchioles and ultimately, after prolonged exposure to the development of adenomas. The observation that MDI particulates do not accumulate in the lung at doses producing lung tumours, together with the lack of chronic inflammation and cytotoxicity, supports the mechanism is via a non-genotoxic, compensatory response of the lung to maintain homeostasis.

Two hypothesis were proposed to explain the carcinogenicity mechanism:

Oncogenesis based on irritation and an epigenetic mechanism,

Oncogenesis resulting from the formation of MDA, which is mutagenic (classified Muta. 2 H341 under regulation (EC) 1272/2008 as mentioned above).

Moreover, in the EU Risk Assessment Report (RAR) of MDI (2005), it was concluded that this substance has no genotoxic properties, although conflicting results were obtained in *in vitro* test systems. *In vivo*, in one micronucleus test, the response in MDI-treated animals did not differ significantly from the control animals. Other studies that have investigated relevant endpoints, such as DNA-adduct formation, did not demonstrate any significant binding after topical or inhalatory exposure to MDI in animals (RAR, 2005). MDI was also evaluated in an *in vivo* mammalian alkaline comet assay (OECD 489) on Wistar rat via inhalation route, with examination of lungs and liver. Negative results were obtained.

Considering the structural similarity between MDI and TODI, a similar toxicological behaviour of TODI can be assumed. Consequently, it is not possible to dismiss the carcinogenic potential of TODI by inhalation route.

Considering the reactivity of TODI and the absence of study on metabolism, it can be considered as a worst case that TODI will be totally metabolised in **TODA** in organisms. Thus, the carcinogenicity data on TODA could be applied to TODI.

TODA has a harmonized classification as Carc. 1B H350 and a classification as Carc. 2B by IARC. In a 14-month study of NTP by oral route with 3,3'-dimethylbenzidine dihydrochloride (CAS 612-82-8, analogue to TODA – NTP, 1991) on F344/N rats, there was a clear evidence of carcinogenic effects on male rats as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, small and large intestine, lungs, and mesothelium. For female rats, there were benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, mammary gland, and lungs. Tumours observed in this study are scattered throughout the entire body, not on one site only, and appear at all doses.

Concerning the genotoxicity endpoint of TODA, even if there is no harmonized classification for this point, data found in literature are equivocal. Such as for TODI, no clear conclusion on genotoxicity mechanism can be drawn.

The issue on the mechanism leading to carcinogenicity (epigenetic or genotoxicity) is thus also raised for TODI as for MDI and TODA.

10.9.2 Comparison with the CLP criteria

For potential classification on carcinogenicity, criteria from CLP-guidance (ECHA, 2017c) were used. Particularly, as there is no data on the substance itself, criteria for classification based on data from similar substances/read across were applied.

A chemical that has not been tested for carcinogenicity may in certain instances be classified as a carcinogen based on tumour data from a structurally similar chemical with which it is predicted to have similar carcinogenic activity. Such an approach must always be based on a robust and transparent argument to support this supposition. There may also be evidence demonstrating similarity in terms of other important factors such as toxicokinetics or mutagenic activity etc. (OECD 2004, 2005, 2007; Guidance on IR&CSA, Section R.6, QSARs and grouping of chemicals).

In the absence of carcinogenicity data, read-across can be used to support a classification for carcinogenicity when the chemical in question is similar to a known or suspected carcinogen (Category 1A, 1B or 2). The similarity between chemicals is considered in terms of structural features, physico-chemical properties and overall toxicological profile.

In general the chemicals will share a common structural element or functional group (i.e., a toxophore) that has been shown to be integral to the underlying mechanism of carcinogenicity for chemicals with this toxiphore in well conducted studies. These toxiphores can be identified through expert judgement or through automated

systems such as (Q)SARs. The read-across should also consider the physico-chemical properties of the chemical and data from other toxicity studies to judge the similarity between the chemicals in terms of bioavailability by relevant routes of exposure and toxicokinetics. The toxicity profile from other studies should also be compared (e.g., acute and repeated-dose toxicity and mutagenicity) and should share similarities in nature and severity. Data from shorter term toxicity studies may be useful, particularly for non-genotoxic carcinogens, to indicate that the chemicals cause the same underlying pathological changes (e.g., hyperplasia), and act via a common mode of action. Any predictions made on the basis of read-across should take into account the totality of data on the chemicals in question, including the physico-chemical properties, toxicological profile, toxicokinetics, structural analogy and the performance of any (Q)SAR models used, in a weight of evidence approach driven by expert judgement. The final decision must be clear, scientifically defensible and transparent.

The specific category depends on the category of the known carcinogen and the degree of confidence in the robustness of the read-across prediction. The category will not be higher than the chemical used to read-across from, but normally may be the same. However a lower category may be applied if the read-across highlights a possible carcinogenic hazard, and thus supports a classification, but there is uncertainty as to the robustness of the read-across prediction or there is evidence, for instance from mechanistic or other studies, that the chemical may be of lower concern for carcinogenicity.

If a chemical is similar to a substance known to be carcinogenic and shares the toxiphore that is considered to be causally related to carcinogenicity, then it is unlikely that there will be sufficient confidence in a prediction of no hazard (for instance based on arguments relating to differences in physico-chemical or steric properties), to justify no classification in the absence of supporting negative experimental data. However, the bioavailability of the toxiphore will need evaluation (Guidance on IR&CSA R.6).

Based on the classification 1B of the hydrolysis product of TODI, TODA, it can be concluded that a classification as category 1B carcinogen could be proposed for TODI.

10.9.3 Conclusion on classification and labelling for carcinogenicity

In this context, a classification as category 1B carcinogen is proposed for TODI according to CLP regulation.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

There are no specific carcinogenicity data on TODI. The hydrolysis product of TODI, TODA (4,4'-bi-o-toluidine, CAS 119-93-7, EC 204-358-0), has a harmonised classification as Carc. 1B (H350) and is classified by IARC in group 2B (possibly carcinogenic to humans). In a 14-month study NTP (1991) study by the oral route with 3,3'-dimethylbenzidine dihydrochloride (CAS 612-82-8), which is an analogue to TODA, on F344/N rats, there was clear evidence of carcinogenic effects on male rats as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, small and large intestine, lungs and mesothelium. In female rats, there were benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, mammary gland and lungs. Tumours observed in this study were scattered throughout the entire body, not localised to one site only, and appeared at all doses.

Considering the reactivity of TODI and the absence of a study on metabolism, the dossier submitter considered as the worst case scenario that TODI will be totally metabolised into TODA in organisms. Therefore, it was concluded that a classification as category 1B carcinogen could be proposed for TODI, based on the classification of its hydrolysis product, TODA. Although read-across to MDI was briefly discussed in the proposal, this was not taken into account in the final classification proposal.

Comments received during consultation

Comments were received from two MSCA and 3 industry organizations. Industry organizations and one MSCA opposed the classification in category 1B based on read-across to TODA, and proposed instead to consider read across to MDI and classification in category 2. The other MSCA considered that the level of detail and lack of robust justification for the read-across applied prevented them from drawing a conclusion on the proposed classification. More detailed analysis and elaboration of the justification was required.

Assessment and comparison with the classification criteria

No data on the adsorption, distribution, metabolism and excretion properties of TODI in mammals were available. In water, TODI hydrolyses rapidly and forms the respective amine, TODA (4,4'-bi-o-toluidine). In a hydrolysis test performed for TODI, the molecule was hydrolysed rapidly (in less than 30 minutes) at 25 and 50 °C both at pH 4 and 9. At pH 7 hydrolysis of 100% was reached within 29 hours at 25 °C and 2.5 hours at 50 °C. The same has been shown to occur also in the case of other similar diisocyanates, like MDI, which forms the carcinogenic 4,4'-methylenedianiline (MDA) by hydrolysis. According to the hydrolysis study communicated in the public consultation (FTI, 2020), the hydrolysis behaviour of MDI and TODI are very similar, showing complete hydrolysis of TODI and MDI in the test system after 1692 min (~28 h) and 1415 min (~24 h), respectively. The relevance of this hydrolysis for the carcinogenicity of TODI, like for the carcinogenicity of MDI (or TDI) is, however, unclear.

The DS considered as a worst case that TODI would be totally hydrolysed to TODA in organisms. Therefore, the classification proposal was based on the data from TODA and the available data from other similar diisocyanates, like MDI and TDI, was ignored. For MDI and TDI toxicokinetic data is available and has been summarised in several reviews, including the EU Risk Assessment Report on MDI and the CORAP evaluation report on TDI (EC, 2005; CoRAP, 2013]. When inhaled, MDI and TDI rapidly conjugate with proteins (which is an essential step in the respiratory sensitisation caused by diisocyanates). When rats were exposed for 4 hours to [^{14}C]-2,4-TDI vapours, the majority of the radiolabelled carbon associated with blood (74-87%) was recovered in the plasma and 97-100% of this radioactivity existed in the form of biomolecular conjugates. Urinary excretion occurred in the form of conjugates and no free TDA was detected. Similarly, MDI-metabolite formation has been shown to proceed primarily via formation of a labile isocyanate glutathione (GSH)-adduct and transfer to more stable adducts with larger proteins. Free diamines have not been typically detected in the blood or urine after inhalation exposure to these diisocyanates (EC, 2005; CoRAP, 2013; Gledhill A. *et al*., 2005) and urinary excretion of MDI and TDI occurs in the form of conjugates. It is, therefore, rather unclear if there are any toxicologically relevant amounts of free MDA/TDA systemically available after inhalation exposure to MDI/TDI or not. After oral exposure, small amounts of free TDA in

urine has been, however, detected in animal experiments. According to (Timchalk *et al.,* 1994) oral exposure of rats to radiolabeled TDI resulted in detectable levels of free or acetylated TDA in urine but the levels were only 2% of the levels detected after a similar dose of TDA. When MDI/TDI were administered orally, protein binding occurred to a lesser degree when compared to the inhalation exposure. Instead, hydrolysis and formation of polyureas was facilitated. After oral dosing, TDI has been shown to polymerise in significant amounts in the acidic environment in the stomach to solid polyureas. This polymerisation reaction limits the absorption of TDI (and TDA formed from TDI) from the gastrointestinal tract.

These data from MDI and TDI do not support the dossier the submitter's worst case assumption that TODI completely hydrolyses to TODA. As has been observed with MDI/TDI, also the 0metabolism of TODI is likely to be more complex than simple hydrolysis to TODA. This is also supported by the available carcinogenicity data on MDI and TDI, which show only mild or no increase in cancer incidence after inhalation exposure. These data were not properly addressed in the dossier submitter proposal but are briefly summarised below.

MDI/pMDI has been studied for carcinogenicity in two inhalation studies. Reuzel *et al.*, (1994) exposed groups of 60 male and 60 female Wistar rats to target concentrations of 0 (controls), 0.2, 1.0 or 6.0 mg/m^3 (analytical value, 0.19, 0.98 or 6.03 mg/m^3) of respirable (particle size, 93.5% < 4.2 µm) polymeric 4,4′ -methylenediphenyl diisocyanate (pMDI) aerosol for 6 h per day, five days a week for two years. The exposure concentrations were selected based on results of a 13-week study. Almost all organs and all grossly observed lesions were examined histologically. Survival rate at 104 weeks of study in males was 38/60, 38/60, 42/60 and 36/60 in the control, low-dose, mid-dose and high-dose groups, respectively and, in females, 41/60, 42/60, 48/60 and 50/60 in control, low-dose, mid-dose and high-dose groups, respectively. In the high-dose group, pulmonary adenomas were found in 6/60 males ($p < 0.05$ by two-sided Fisher's exact test) and 2/59 females, and pulmonary adenocarcinoma was found in 1/60 males. No lung tumours were found in other dose groups. Accumulation of alveolar macrophages containing pMDI-associated refractile yellowish material, localized fibrosis, alveolar duct epithelialisation and increased incidences of calcareous deposits and localized alveolar bronchiolisation were observed in the lungs of the high-dose group.

Hoymann *et al.* (1995) conducted a chronic inhalation study with 99.5% pure monomeric 4,4'-MDI. Female Wistar rats (80 per exposure group) were exposed (whole body) to MDI aerosol at 0.23, 0.70, or 2.05 mg/m^3 (MMAD about 1 µm) for 17 h per day, 5 days per week, for up to 24 months. A separate group of 20 animals per exposure level was examined histopathologically at 12 months. Smaller numbers of animals were assessed at various time points for lung function and for examination of BAL fluid (cell counts and protein and enzyme determinations). Statistically significant concentration-related pulmonary lesions included (1) an increase in focal/multifocal alveolar and bronchioalveolar hyperplasia, (2) interstitial fibrosis and (3) accumulation of particle-laden and pigmented macrophages. Alveolar cell hyperplasia, considered preneoplastic, exhibited a concentration–response trend, with the incidence reaching statistical significance in the high-exposure group. These effects correlated with pulmonary function deficits (FEF25 [forced expiratory flow from 25% of the forced vital capacity (FVC)] and carbon monoxide diffusion), particularly in the high-exposure group. All groups exhibited significantly increased relative lung weights at all time periods (more than 60% at 20 months), with significant increases in hydroxyproline in BAL fluid (more than 70% at 12 months). In contrast to the results reported by Reuzel *et al.* (1994b) for pMDI, there was no apparent

effect of monomeric MDI on nasal tissues at any exposure level. In one high-dose animal, a bronchiolo-alveolar adenoma was observed. Because of the concentration-related lung effects, 0.23 mg/m^3 is considered a LOAEL. There is no NOAEL in this study.

Mechanisms of lung tumours caused by MDI/pMDI in rats has been proposed to be non-genotoxic and related to the increase in regenerative proliferation of type-II cells resulting in pre-neoplastic changes, which is a known chronic reaction of rat lung to irritating substances. *In vivo* Comet assay performed in accordance with OECD TG 489 to assess the potential of aerosolised 4,4'-MDI to cause DNA damage in lung, liver or stomach following a single inhalation exposure remained negative, which supported the role of mechanisms other than genotoxicity in the lung carcinogenesis caused by MDI.

Toluene diisocyanate (TDI) has also been the subject of carcinogenicity inhalation studies and as is the case with MDI, has a harmonised classification in category 2 for carcinogenicity. TDI data were not considered by the dossier submitter in their proposal despite TDI belonging to the same group of aromatic diisocyanates and forming the carcinogenic amine (TDA) by hydrolysis (similarly to MDI and TODI). Carcinogenicity data on TDI has been, however, summarised on other occasions e.g. in the CORAP evaluation report on TDI (EC, 2005). Inhalation exposure of male and female rats to TDI at levels of 0.05 and 0.15 ppm (0.36 and 1.1 mg/m^3) 6 h/day, 5 days/week, two years did not provide any evidence of carcinogenicity (EC, 2005), whereas increased frequencies of several types of tumours (e.g. subcutaneous fibromas and sarcomas in male and female rats, pancreatic acinar cell adenomas in male rats, pancreatic islet cell adenomas, neoplastic nodules of the liver and fibroadenomas in female rats; and mammary gland hemangiomas, hemangiosarcomas, hepatocellular adenomas in female mice) were observed when rats and mice were exposed to TDI in corn oil by gavage (0, 60, 120 mg/kg bw/day female rats; 0, 30, 60 mg/kg bw/day male rats; 0, 120, 240 mg/kg bw/day male mice; 5 days/week, 105 weeks (mice) or 106 weeks (rats)). The pattern of multifocal tumours following oral exposure was similar to the carcinogenic responses produced by the hydrolysis product TDA. However, the sample administered to rats also contained breakdown and reaction products of TDI, which questions the validity of the study. Therefore, in the CoRAP evaluation report on TDI (EC, 2005) it is concluded that "*the results of the studies using oral administration are compromised by severe deficiencies in test substance handling that led to the fact that the sample administered also contained other unidentified breakdown and reaction products of TDI, possibly including TDA. In addition, the addition of TDI directly into the acidic environment of the stomach, bypassing the oral cavity, is an unrealistic exposure scenario which leads to generation of the diamine which would not occur in normal handling and use*."

There is no oral carcinogenicity data on MDI. The MDI hydrolysis product, **MDA,** has been shown to cause an increase in liver and thyroid tumour incidence when administered via gavage. Liver tumours have been considered to be caused by the genotoxic MoA but for thyroid cancers there are plausible non-genotoxic mechanisms based on hormonal disruption due to liver damage (ECHA, 2015).

Based on the data on toxicokinetics and carcinogenicity of the similar diisocyanates MDI and TDI, the dossier submitter worst case scenario that TODI would be totally metabolised in TODA in organisms and that the classification could be based on the carcinogenicity data on TODA is not considered scientifically justified. However, considering the structural similarity and likely similar behaviour in the body, read across to MDI/TDI can be justified.

Comparison with the criteria

In the case of TODI no human data exists and therefore Category 1A is not applicable.

Category 1B is indicated in the case of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in at least two species or in two independent studies in one species. If there are no data on the substance itself, criteria for classification based on data from similar substances/read across can be applied. Such an approach must always be based on a robust and transparent argument to support this supposition. The hydrolysis product of TODI, TODA, is classified as a category 1B carcinogen on the basis of animal data showing increases in tumours in multiple organs after oral exposure. Although TODI is known to form TODA by hydrolysis, it is not clear how much TODA is formed *in vivo*. The data from other similar diisocyanates, MDI and TDI, suggests that after inhalation, diisocyanates are rapidly conjugated with proteins and hydrolysis of diisocyanates is of minor importance. Although after oral exposure protein binding is lower when compared to inhalation exposure, polymerization to solid polyureas have been shown to occur in stomach, which reduces the amount of free diisocyanate and corresponding amine available for absorption. Because of this, direct read-across to TODA and classification as Carc. 1B based on the carcinogenicity data on TODA is not considered scientifically justified.

The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. If there are no data on the substance itself, criteria for classification based on data from similar substances/read across can be applied. According to CLP, the category will not be higher than the chemical used to read-across from, but normally may be the same. However, a lower category may be applied if the read-across highlights a possible carcinogenic hazard, and thus supports a classification, but there is uncertainty as to the robustness of the read-across prediction or there is evidence, for instance from mechanistic or other studies, that the chemical may be of lower concern for carcinogenicity.

The diisocyanates MDI and TDI have been classified as Carc. 2 on the basis of experimental evidence. pMDI caused an increase in lung tumours after inhalation exposure of rats whereas TDI remained negative. The MoA for MDI induced lung tumours is likely to be related to the irritation and regenerative proliferation of type-II cells. In an oral carcinogenicity study with TDI, increased frequencies of several types of tumours were seen. Multifocal tumours following oral exposure to TDI could be explained by the formation toluene diamine (TDA) from TDI in the study conditions. However, there was a suspicion on the presence of TDA already in the substance administered to the rats and on the formation of higher levels of TDA due to the use of gavage route of administration. Both MDI and TDI have harmonised classifications as category 2 carcinogens. Considering the structural similarity and likely similar behaviour in the body, read across to MDI/TDI is justified. Based on the read across to MDI/TDI, RAC concludes that **TODI warrants classification as Carc. 2 (H351).**

10.10 Reproductive toxicity

Not relevant for this dossier

10.11 Specific target organ toxicity-single exposure

Not relevant for this dossier

10.12 Specific target organ toxicity-repeated exposure

Not relevant for this dossier

10.13 Aspiration hazard

Not relevant for this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not relevant for this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not relevant for this dossier

13 ADDITIONAL LABELLING

According to the CLP regulation, Annex II, section 2.4, the following special rule for supplemental label elements shall apply for mixtures containing m-XDI:

"Unless already identified on the label of the packaging, mixtures containing isocyanates (as monomers, oligomers, prepolymers, etc., or as mixtures thereof) shall bear the following statement:

EUH204 — 'Contains isocyanates. May produce an allergic reaction."

14 REFERENCES

Agius R.M. (2000): Why are some low-molecular-weight agents asthmagenic. Occupational Medicine (Pittsburgh, PA) 15 (2), 369-384

Anonymous (1996). Mutagenicity Test Data of Existing Chemical Substances. Japan Chemical Industry Ecology Toxicology & Information Center, Japan (JETOC).

Anonymous (1998a). TODI: Micronucleus Test in the Mouse.

Anonymous (1998b). TODI: Twenty-Eigth Day Repeated Dose Oral (gavage) Toxicity Study in the Rat.

Anonymous (1999a). L5178Y TK +/- Mouse Lymphoma Assay.

Anonymous (1999b). TODI: In vivo liver unscheduled DNA synthesis (UDS) assay.

Aoyama K., Huang J., Ueda A., and Matsushita T. (1994): Provocation of respiratory allergy in guinea pigs following inhalation of free toluene diisocyanate. Archives of Environmental Contamination and Toxicology 26 (3), 403-407. DOI: 10.1007/BF00203570 (last accessed 2016-09-19)

Arts J.H.E., de Jong W.H., van Triel J.J., Schijf M.A., de Klerk A., van Loveren H., and Kuper C.F. (2008): The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106 (2), 423-434. DOI: 10.1093/toxsci/kfn199 (last accessed 2016-09-19)

Ban M., Morel G., Langonné I., Huguet N., Pépin E., and Binet S. (2006): TDI can induce respiratory allergy with Th2-dominated response in mice. Toxicology 218 (1), 39-47. DOI: 10.1016/j.tox.2005.09.013 (last accessed 2016-09-19)

Bayer (1995): Diphenylmethane-4,4'-diisocyanate (MDI-monomer). Evaluation of respiratory sensitization in guinea-pigs following brief high-level inhalation induction exposure and challenge with ramped MDI

concentrations. Report no. T1058323, III project 121-EU-MTX, III Report 11184, date: 1995-05-30. Bayer AG, Department of Toxicology. International Isocyanate Institute, unpublished

Botham P.A., Hext P.M., Rattray N.J., Walsh S.T., and Woodcock D.R. (1988): Sensitisation of guinea pigs by inhalation exposure to low molecular weight chemicals. Toxicology Letters 41 (2), 159-173. DOI: 10.1016/0378-4274(88)90089-6 (last accessed 2016-09-19)

de Jong W.H., Arts J.H.E., de Klerk A., Schijf M.A., Ezendam J., Kuper C.F., and van Loveren H. (2009): Contact and respiratory sensitizers can be identified by cytokine profiles following inhalation exposure. Toxicology 261 (3), 103-111. DOI: 10.1016/j.tox.2009.04.057 (last accessed 2016-09-19)

Dearman R.J. and Botham P.A. (1990): Inhalation exposure to respiratory sensitising chemicals down-regulates guinea pig IgE and pulmonary responses. International Archives of Allergy and Applied Immunology 92 (4), 425-432. DOI: 10.1159/000235175 (last accessed 2016-09-19)

Dik S., Ezendam J., Cunningham A.R., Carrasquer C.A., van Loveren H., and Rorije E. (2014): Evaluation of In Silico Models for the Identification of Respiratory Sensitizers. Toxicological Sciences 142 (2), 385-394. DOI: 10.1093/toxsci/kfu188

Ebino K., Ueda H., Kawakatsu H., Shutoh Y., Kosaka T., Nagayoshi E., Lemus R., and Karol M.H. (2001): Isolated airway exposure to toluene diisocyanate results in skin sensitization. Toxicology Letters 121 (1), 79-85. DOI: 10.1016/S0378-4274(01)00325-3 (last accessed 2016-09-19)

ECHA (2016): Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint-specific guidance. Draft version 5.0, date: 2016-06. European Chemicals Agency. http://echa.europa.eu/documents/10162/13643/ir_csa_r7a_r7-3_caracal_draft_en.pdf (last accessed 2016-09-19)

ECHA (2017a): Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0, date: 2017-07. European Chemicals Agency. European Chemicals Agency, Helsinki, Finland. https://echa.europa.eu/documents/10162/23036412/clp_en.pdf (last accessed 2016-09-19)

ECHA (2017b): Read-across assessment framework. ECHA-17-R-01-EN, date: 2017-03. European Chemicals Agency (ECHA), Helsinki, Finland. DOI: 10.2823/619212 (last accessed 2018-06-21)

Enoch S.J., Ellison C.M., Schultz T.W., and Cronin M.T.D. (2011): A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. Critical Reviews in Toxicology 41 (9), 783-802. DOI: 10.3109/10408444.2011.598141

Enoch S.J., Roberts D.W., and Cronin M.T.D. (2009): Electrophilic reaction chemistry of low molecular weight respiratory sensitizers. Chemical Research in Toxicology 22 (8), 1447-1453. DOI: 10.1021/tx9001463

Enoch S.J., Roberts D.W., Madden J.C., and Cronin M.T. (2014): Development of an in silico profiler for respiratory sensitisation. Alternatives to laboratory animals : ATLA 42 (6), 367-375

European Chemicals Bureau (2005). EU Risk Assessment Report: Methylenediphenyl diisocyanate (MDI). EU Riske Assessment Report, 3rd Priority List, Vol. 59, 2005. Report no.: Volume 59. Report date: 2005-01-01.

European Parliament and Council (2008): Regulation (EC) no. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 (last amended by Commission Regulation (EU) 2015/491 of 23 March 2015). European Union, Brussels. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1272-20160101&from=EN (last accessed 2016-09-19)

Feron VJ, Kittel B, Kuper CF, Ernst H, Rittinghausen S, Muhle H, Koch W, Gamer A, Mallett AK & Hoffmann HD (2001). Chronic pulmonary effects of respirable methylene diphenyl diisocyanate (MDI) aerosol in rats: combination of findings from two bioassays. Arch Toxicol.75: 159-175.

Gagnaire F., Ban M., Cour C., Micillino J.C., Bonnet P., and Hettich D. (1997): Role of tachykinins and neutral endopeptidase in toluene diisocyanate-induced bronchial hyperresponsiveness in guinea pigs. Toxicology 116 (1-3), 17-26. DOI: 10.1016/S0300-483X(96)03517-2 (last accessed 2016-09-20)

Gagnaire F., Ban M., Micillino J.C., Lemonnier M., and Bonnet P. (1996): Bronchial responsiveness and inflammation in guinea-pigs exposed to toluene diisocyanate: A study on single and repeated exposure. Toxicology 114 (2), 91-100. DOI: 10.1016/S0300-483X(96)03415-4 (last accessed 2016-09-20)

German CA (2016): Annex XV report. Proposal for a restriction. Substance name(s): Diisocyanates, date: 2016-10-06. https://echa.europa.eu/documents/10162/0013a374-b200-7486-6111-869c200f9a66 (last accessed 2018-06-21)

Hoymann H.G., Buschmann J., and Heinrich U. (1995): Untersuchungen zur chronischen Toxizität/Kanzerogenität von 4,4'-Methylendiphenyl-Diisocyanat (MDI) [Studies on the chronic toxicity/carcinogenicity of 4,4'-methylenediphenyl-diisocyanate (MDI)]. Forschungsbericht 116 06 084, date: 1995-09-01. Fraunhofer-Institut für Toxikologie und Aerosolforschung. Umweltbundesamt (UBA)

Huang J., Aoyama K., and Ueda A. (1993a): Experimental study on respiratory sensitivity to inhaled toluene diisocyanate. Archives of Toxicology 67 (6), 373-378. DOI: 10.1007/BF01977397 (last accessed 2016-09-20)

Huang J., Wang X.P., Chen B.M., Zhou X.J., and Matsushita T. (1993b): Dose-response relationships for chemical sensitization from TDI and DNCB. Bulletin of Environmental Contamination and Toxicology 51 (5), 732-739. DOI: 10.1007/BF00201652 (last accessed 2016-09-20)

IARC (1987) Overall Evaluations Of Carcinigenicity : An Updating of IARC Monographs Volumes 1 to 42. Supplement 7. Lyon, France.

IARC (1999) VOLUME 71 Re-Evaluation Of Some Organic Chemicals, Hydrazine And Hydrogen Peroxide. IARC Lyon France

Karol M.H. (1983): Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicology and Applied Pharmacology 68 (2), 229-241. DOI: 10.1016/0041-008X(83)90007-8 (last accessed 2016-09-20)

Laky V. (2009): Determination of the hydrolysis of TODI as a function of pH (preliminary test), date: 2009-08-17. LAB Research Ltd.,. Nippon Soda Co. L.S.-O.B., 2-1 Ohtemachi 2-chome chiyoda-ku J-100-8165 TOKIO, Japan, unpublished

Lalko J.F., Kimber I., Dearman R.J., Api A.M., and Gerberick G.F. (2013): The selective peptide reactivity of chemical respiratory allergens under competitive and non-competitive conditions. Journal of immunotoxicology 10 (3), 292-301. DOI: 10.3109/1547691x.2012.725784

Matheson J.M., Johnson V.J., and Luster M.I. (2005a): Immune mediators in a murine model for occupational asthma: Studies with toluene diisocyanate. Toxicological Sciences 84 (1), 99-109. DOI: 10.1093/toxsci/kfi051 (last accessed 2016-09-20)

Matheson J.M., Johnson V.J., Vallyathan V., and Luster M.I. (2005b): Exposure and immunological determinants in a murine model for toluene diisocyanate (TDI) asthma. Toxicological Sciences 84 (1), 88-98. DOI: 10.1093/toxsci/kfi050 (last accessed 2016-09-19)

Niimi A., Amitani R., Yamada K., Tanaka K.I., and Kuze F. (1996): Late respiratory response and associated eosinophilic inflammation induced by repeated exposure to toluene diisocyanate in guinea pigs. Journal of Allergy and Clinical Immunology 97 (6), 1308-1319. DOI: 10.1016/S0091-6749(96)70200-2 (last accessed 2016-09-20)

NTP (1991) *Toxicology and carcinogenesis studies of 3,3'-dimethylbenzidine dihydrochloride in F344/N rats, NTP n°390*

OECD (2012): The adverse outcome pathway for skin sensitisation initiated by covalent binding to proteins. Part 1: Scientific evidence. Document ENV/JM/MONO(2012)10/PART1, date: 2012-04-05. Organisation for Economic Co-operation and Development (OECD), Paris. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282012%2910/part1&doclanguage=en (last accessed 2016-09-20)

Pauluhn J. (1994): Assessment of chemicals for their potential to induce respiratory allergy in guinea pigs: A comparison of different routes of induction and confounding effects due to pulmonary hyperreactivity. Toxicology in Vitro 8 (5), 981-985. DOI: 10.1016/0887-2333(94)90231-3 (last accessed 2016-09-20)

Pauluhn J. and Mohr U. (1998): Assessment of respiratory hypersensitivity in guinea pigs sensitized to toluene diisocyanate: A comparison of sensitization protocols. Inhalation Toxicology 10 (2), 131-154. DOI: 10.1080/089583798197790 (last accessed 2016-09-20)

Rattray N.J., Botham P.A., Hext P.M., Woodcock D.R., Fielding I., Dearman R.J., and Kimber I. (1994): Induction of respiratory hypersensitivity to diphenylmethane-4,4'-diisocyanate (MDI) in guinea pigs. Influence of route of exposure. Toxicology 88, 1-3. DOI: 10.1016/0300-483X(94)90108-2 (last accessed 2016-09-20)

Reuzel et al.PGJ, Arts JHE, Lomax LG, Kuijpers MHM, Kuper CF, Gembardt C, Feron VJ and Löser E. (1994). Chronic inhalation toxicity and carcinogenicity study of respirable polymeric methylene diphenyl diisocyanate (polymeric MDI) aerosol in rats. Fund.Appl.Toxicol. 22: 195-210.

Safepharm (1998): TODI: Magnusson & Kligman maximisation study in the guinea pig. Report no.: 235/224, date: 1998-02-26. Safepharm Laboratories Ltd. Nippon Soda Co. Ltd., unpublished

SCC (2010): Toxicokinetic assessment of TODI. Report no.: 309-028. SCC. Nippon Soda Co. Ltd., unpublished

Additional references

Prüfbericht FTI-Auftragsnummer 802505, Freudenberg Technology Innovation SE& Co.KG, 15.05.2020

European Union Risk Assessment Report, 4,4'-methylenediphenyl diisocyanate (MDI), EC No 202-966-0, Volume 59, 2005

CoRAP Substance Evaluation Report, m-tolylidene diisocyanate (TDI), EC No. 247-722-4, November 2013

Gledhill A *et al*., Absorption, distribution, metabolism and excretion of an inhalation dose of [14C] 4,4-methylenediphenyl diisocyanate in the male rat, Xenobiotica, March 2005; 35(3): 273-292

ECHA/RAC 2015 APPLICATION FOR AUTHORISATION: ESTABLISHING A REFERENCE DOSE RESPONSE RELATIONSHIP FOR CARCINOGENICITY OF TECHNICAL MDA. Helsinki, 26 March 2015. RAC/32/2015/11 rev 1

Timchalk *et al* (1994) Route-Dependent Comparative Metabolism of [14C]Toluene 2,4-Diisocyanate and [14C]Toluene 2,4-Diamine in Fischer 344 Rats, Toxicology and Applied Pharmacology, Volume 124, Issue 2, February 1994, Pages 181-19

15 LIST OF ABBREVIATIONS

AB: Antibodies

ADME: Absorption, distribution, metabolism, and excretion

AE: Aerosol

AHR: Airway hyperresponsiveness

AOP: Adverse outcome pathway

BAL(F): Bronchoalveolar lavage (fluid)

BHR: Bronchial hyperresponsiveness

BT: Biuret

CLH: Harmonised classification and labelling

CLP: Classification, labelling, and packaging

DO: Dog

DS: Dossier submitter

DSC: Differential scanning calorimetry

DH: Dunkin-Hartley

ECHA: European Chemicals Agency

ERR: Exposure-Reponse-Relationship

ESH: English smooth-hair

F: Female

FEF_{25-75}: Forced expiratory flow between 25 and 75% of FVC

FEV_1: Forced Expiratory Volume in one second

FEV_1%: FEV_1/FVC x 100

FVC: Forced vital capacity

GLP: Good laboratory practice

GP: Guinea pig

GPSA: Guinea pig serum albumin

HDI: Hexamethylene diisocyanate

HH: Human health

HMDI: “Hydrated MDI”, 4'-methylenedicyclohexyl diisocyanate

HO: Head-only

IC: Isocyanurate

IDE: Intradermal

IF: Inflammation

IgE/IgG: Immunoglobulin E/G

INA: Intranasal

INH: Inhalation

IPDI: Isophoronediisocyanate

IPE: Intraperitoneal

IR & CSA: Information requirements and chemical safety assessment

ITR: Intratracheal

IUCL: Only IUCLID summary available

IVE: Intravenous

JEM: Job exposure matrix

LLNA: Local lymph node assay

LOD: Limit of detection

MDI: 4,4'-Methylenediphenyl-diisocyanate

M: Male

MIE: Molecular initiating event

MMF: Maximum mid-expiratory flow

MO: Mouse

NCO: Isocyanate functional group

NDI: 1,5-Naphthylene-diisocyanate

NO: Nose-only

n.s.: Not significant

OA: Occupational asthma

OR: Odds Ratio

OECD: Organization for Economic Co-Operation and Development

OVA: Ovalbumin

PEF(R): Peak expiratory flow (rate)

PHDI: Polymeric HDI

PIPDI: Polymeric IPDI

PMDI: Polymeric MDI

PR: Prevalence ratio

PU: Polyurethane

QSAR: Quantitative Structure-Activity Relationship(s)

RA: Rat

RB: Rabbit

REACH: Registration, evaluation, authorisation and restriction of chemicals

RF: Respiratory function

RR: Relative Risk

RS: Respiratory sensitisation

SCU: Subcutaneous

SS: Skin sensitisation

TDI: Toluyenediisocyanate, mixed isomers, isomer ratio 80:20 (2,4:2,6)

TDI_{UC}: TDI of unclear composition

TMI: Toluylenemono-isocyanate

m-TMXDI: 1,3-Bis(1-isocyanato-1-methyl-ethyl)benzene

TODI: 3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate

TOE: Toepad inoculation

TOP: Topical

TWA: Time-weighted average

VP: Vapour

WB: Whole-body