Committee for Risk Assessment

RAC

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

Adopted

18 March 2021

18 March 2021

CLH-O-0000006965-60-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate**

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

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

The proposal was submitted by **Germany** and **France** and received by RAC on **11 February 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany and France have submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 March 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **8 May2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Tiina Santonen**

Co-Rapporteur, appointed by RAC: **Veda Varnai**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	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	202-112-7	91-97-4	Carc. 1B Resp. Sens. 1 Skin Sens. 1A	H350 H334 H317	GHS08 Dgr	H350 H334 H317		Skin Sens. 1A; H317: C ≥0.001%	
RAC opinion	TBD	3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate	202-112-7	91-97-4	Carc. 2 Resp. Sens. 1 Skin Sens. 1A	H351 H334 H317	GHS08 Dgr	H351 H334 H317		Skin Sens. 1A; H317: C ≥0.001%	
Resulting Annex VI entry if agreed by COM	TBD	3,3'-dimethylbiphenyl-4,4'-diyl diisocyanate	202-112-7	91-97-4	Carc. 2 Resp. Sens. 1 Skin Sens. 1A	H351 H334 H317	GHS08 Dgr	H351 H334 H317		Skin Sens. 1A; H317: C ≥0.001%	

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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	SS	
			IDE	TDI-GPSA			8				RF	
			INH	TDI-GPSA/ TMI-GPSA			12					
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					
							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).

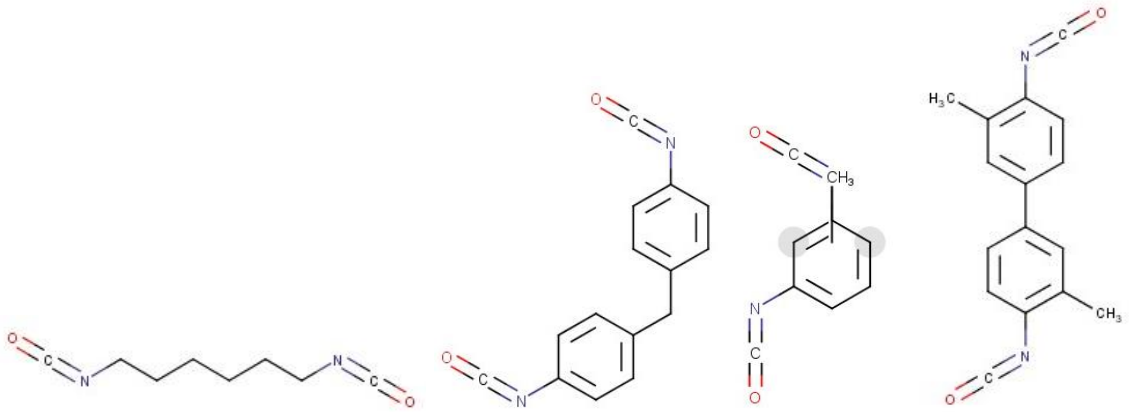

Figure. *The structures of HDI, MDI, TDI and TODI, respectively, from left to right*.

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.

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).

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 metabolic activation and 6, 8, 10, 12, 14 µg/mL with metabolic activation (3h exposure)	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).	Anonymous (1999a)
Similar to OECD test guideline 473	TODI with a purity of 99.8%. The	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,	Slightly positive results were obtained with metabolic	Anonymous / JETOC (1996)

	vehicle was DMSO.	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.	activation at 0.6 mg/mL.	
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 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)

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.**

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).**

Additional references

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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

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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

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).