CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,3-Bis(1-isocyanato-1-methylethyl)benzene;

[m-TMXDI]

EC Number: **220-474-4**

CAS Number: **2778-42-9**

Index Number: **n.a.**

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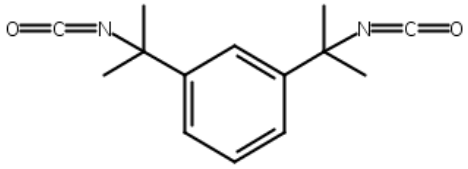
CONTENTS

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)	1,3-bis(1-isocyanato-1-methylethyl)benzene
Other names (usual name, trade name, abbreviation)	meta-Tetramethylxylylenediisocyanate (m-TMXDI)
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	220-474-4
EC name (if available and appropriate)	1,3-bis(1-isocyanato-1-methylethyl)benzene
CAS number (if available)	2778-42-9
Other identity code (if available)	-
Molecular formula	$C_{14}H_{16}N_2O_2$
Structural formula	
SMILES notation (if available)	CC(C)(N=C=O)c1cccc(c1)C(C)(C)N=C=O
Molecular weight or molecular weight range	244.29 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)	-

1.2 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)
1,3-bis(1-isocyanato-1-methylethyl)benzene EC No. 220-474-4 CAS No. 2778-42-9	80-100	-	Skin Irrit. 2 (H315), Skin Sens. 1/1A (H317), Eye Irrit. 2 (H319), Acute Tox. 1 (H330), Resp. Sens. 1 (H334), STOT SE 3 (H335), STOT RE 1 (H372, Inhalation), 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 m-TMXDI

	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	1,3-bis(1-isocyanato-1-methylethyl)benzene; [m-TMXDI]	220-474-4	2778-42-9	Resp. Sens. 1 Skin Sens. 1A	H334 H317	GHS08 Dgr	H334 H317			
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	Hazard class not assessed in this dossier	No
Carcinogenicity		
Reproductive toxicity		
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure		
Aspiration hazard		
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Not applicable

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.

5 IDENTIFIED USES

A summary of the information available on ECHA's public website (accessed 2017-06-29) is given below[1].

5.1 General

This substance is manufactured and/or imported in the European Economic Area in 100 - 1000 tonnes per year. This substance is used at industrial sites and in manufacturing.

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

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. ECHA has no public registered data indicating whether or into which articles the substance might have been processed.

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.

[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.

5.6 Uses at industrial sites

This substance is used in the following products: Polymers. This substance has an industrial use resulting in manufacture of another substance (use of intermediates). ECHA has no public registered data on the types of manufacture using this substance. This substance is used in the following activities or processes at workplace: Transfer of chemicals between vessels/large containers, closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, batch processing in synthesis or formulation with opportunity for exposure, mixing in open batch processes, transfer of substance into small containers and laboratory work. Release to the environment of this substance is likely to occur from industrial use: manufacturing of the substance, as an intermediate step in further manufacturing of another substance (use of intermediates) and for thermoplastic manufacture.

5.7 Manufacture

This substance is used in the following activities or processes at workplace: transfer of chemicals between vessels/large containers, closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, batch processing in synthesis or formulation with opportunity for exposure, mixing in open batch processes, transfer of substance into small containers and laboratory work. Release to the environment of this substance is likely to occur from industrial use: manufacturing of the substance, as an intermediate step in further manufacturing of another substance (use of intermediates) and for thermoplastic manufacture.

6 DATA SOURCES

This report has been created based on the data submitted by the lead registrant in the REACH registration dossier for m-TMXDI. In addition, further relevant data on m-TMXDI and related diisocyanates (cf. section 10.6) were retrieved as part of a general literature search in the context of the restriction proposal for diisocyanates recently submitted to ECHA by the Dossier Submitter (DS).

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	Liquid	-
Melting/freezing point	4 °C (melting range marked by onset and endset of melting peak: 4-12 °C)	Experimental result [OECD Guideline 102 (Melting point/Melting Range)]
Boiling point	DSC: 249.2 °C (1 atm); ebulliometer: 249.4 °C (1 atm)	Experimental result [OECD Guideline 103 (Boiling point/boiling range): DSC and ebulliometer]

Property	Value	Comment (e.g. measured or estimated)
Relative density	1.0742 (at 20 °C); 1.0698 (at 25 °C)	Experimental result [OECD Guideline 109 (Density of Liquids and Solids): oscillating densitometer]
Vapour pressure	0.0029 mm Hg (0.386 Pa) at 25 °C	Experimental result [OECD Guideline 104 (Vapour Pressure Curve): effusion method]
Surface tension	38.6 mN/m (at 20 °C)	Experimental result [OECD Guideline 115 (Surface Tension of Aqueous Solutions): Ring Method]
Water solubility	N.a.; hydrolytically unstable at pH 4, 7, and 9 (half-life less than 12 hours)	-
Partition coefficient n-octanol/water	Estimated log Kow: 4.74; Estimated log Kow values of hydrolytic products: ▪ 3.53 for 1,3-bis(2-propan-2-yl)urea ▪ 1.89 for tetramethyl-m-xylylene diamine	Estimated by calculation [Partition coefficient estimation using KOWWIN v1.67 of EPISuite program, EPIWEB v 4.0]
Granulometry	N.a. (liquid)	-
Stability in organic solvents and identity of relevant degradation products	N.a. (stability in organic solvents is not a critical property of the substance)	-
Dissociation constant	N.a. (hydrolytically unstable)	-
Viscosity	Dynamic: 18.2 mPas (at 25 °C)	Experimental result [method equivalent or similar to OECD Guideline 114: rotational viscometer]

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 m-TMXDI are available. In the registration dossier, the lead registrant has provided some estimates based on the structure and physico-chemical properties, which, together with the DS comments and slight editorial amendments are presented in Table 6 below.

Table 6 Estimation of ADME properties by the lead registrant for m-TMXDI

Property	Estimate by Registrant	DS Comment
Hydrolysis and metabolism	In the presence of water, m-TMXDI has been shown to hydrolyse and form urea or polyurea, as well as tetramethyl-m-xylylene diamine (under conditions of high dispersion and low concentration). Regardless of the exposure route, it is therefore possible that both the parent compound and its hydrolysis products are present in the organism.	A hydrolysis study according to OECD TG 111 is available (only as an IUCLID summary in the registration dossier). Depending on pH and temperature, the reported rate constants and estimated half-lives were as follows (Wooley and Mulley, 2003): - pH 1.2, 37 ± 0.5 °C: "almost instant degradation in the media, with only 3.45 % of the fortified concentration remaining at the time zero analysis", - pH 4, 25 ± 0.5 °C: 1.692 h^{-1}/0.410 h, - pH 7, 25 ± 0.5 °C: 1.9044 h^{-1}/0.364 h, - pH 9, 37 ± 0.5 °C: 2.0664 h^{-1}/0.336 h. At pH ≥ 4 (relevant for contact via the skin or by inhalation) after about 20-25 min still half of the original diisocyanate was present in the media (25 % after ca. 40-50 min, 12.5 % after ca. 80-90 min etc.). This provides a sufficient time window for the initial steps of sensitisation to take place. In addition reactions of m-TMXDI with proteins to form a protein-hapten complex compete with hydrolysis due to moisture on the skin/within the respiratory tract, and thus the fraction effectively available for sensitisation could be greater than suggested by the above figures. The registrant did not provide data to support his analysis of metabolism which, however, appears plausible based on experience with other diisocyanates.
Absorption via inhalation and the dermal route	m-TMXDI and the corresponding urea both have molecular weights below 500 and an estimated log Pow > 4, suggesting that transfer into the epidermis from the stratum corneum of skin and direct uptake across the respiratory tract by passive diffusion would be limited (see section R.7.12.2.1 of REACH guidance document R7.C). Inhalatory absorption via micellar solubilisation could nevertheless occur. The tetramethyl-m-xylylene diamine on the other hand has an estimated log Pow of 1.89, which suggests a higher direct absorption potential.	The statements of the registrant correctly reflect the content of the guidance which, however, also notes that "If the substance has been identified as a skin sensitiser then, provided the challenge application was to intact skin, some uptake must have occurred although it may only have been a small fraction of the applied dose." The Molecular Initiating Event (MIE) of sensitisation, i.e. binding of the low-molecular weight chemical hapten to protein to form a protein-hapten complex, may however occur already at the site of entry. Knowledge about the systemic distribution (and eventual elimination) is therefore not needed for deciding qualitatively on the sensitisation potential of the diisocyanates.
Bioaccumulation	Once absorbed, neither m-TMXDI nor the hydrolysis products are expected to bioaccumulate significantly, based on the results of the fish bioconcentration study which yielded a BCF below 10.	The available bioaccumulation test reports BCFs of < 1.2-2.7 and 1-5.7 at concentrations of 0.1 and 1.0 mg/L (Sudo, 1985). Moreover, in the view of the DS, due to its hydrolysability and in line with the experience gained with other diisocyanates, m-TMXDI is unlikely to possess a potential for bioaccumulation.
Excretion	Other polyisocyanates such as MDI or TDI have been shown to conjugate with albumin in the circulatory system, with excretion via urine occurring within a few hours. Depending on exposure, a pool of isocyanate-conjugated albumin may persist in the circulatory system and reach a steady-state.	The registrant's statement is correct, however, albumin adducts are not the only adducts observed with diisocyanates, cf. e.g. (Sabbioni et al., 2016).

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 m-TMXDI, the substance addressed by this dossier, data with respect to RS are scarce.

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 presenting the few available substance-specific data for m-TMXDI which on their own do not suffice to classify it as a respiratory sensitiser. In a second step, these data are then complemented via 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 m-TMXDI. 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))

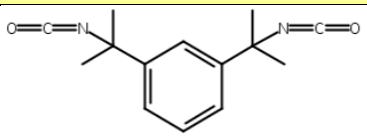
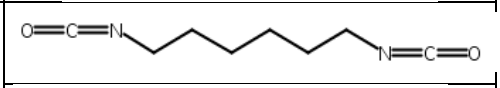
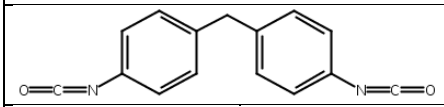
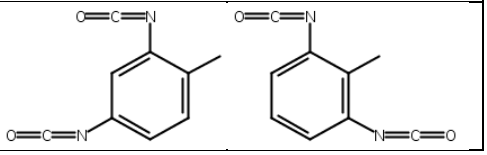
The approach relates to RAAF Scenario 6 (human health), i.e. the read-across hypothesis for the category is based on different compounds which have qualitatively similar properties, with no relevant variations in properties observed among source substances and the same strength predicted for the target 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 m-TMXDI has been characterised above. Table 7 provides information on the identity and harmonised classification of the target substance as well as the category source substances HDI, MDI, and TDI.

Table 7: Overview of target and category source substances used for read-across to m-TMXDI

EC Name; trivial name used in this report	EC No. CAS no.	CLH for sensitisation (Annex VI to CLP)	Structure
1,3-bis(1-isocyanato-1-methylethyl)benzene; m-TMXDI	220-474-4 2778-42-9	-	
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 7, 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

As will be illustrated in the following sections, 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.

[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.

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 1.

10.6.2.6 AE C.5 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 1.

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 7 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.

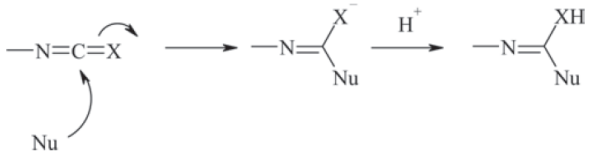


Figure 1: Acylation reaction for isocyanates (X = oxygen). Reproduced from (Enoch et al., 2011)

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 between different proteins which may result in a more marked change of conformation.

The potential reactivity of the diisocyanate source substances given in Table 7 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 7 above, the DS 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 is not addressed in this dossier).

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

The DS 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, the DS 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. The DS 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 the DS as follows:

- Identify all studies in humans and animals for m-TMXDI, 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, first for m-TMXDI, then for the three source substances HDI, MDI, and TDI.
- Filter animal data for relevance according to predefined criteria (cf. section 10.6.5).
- Evaluate and present the relevant animal data, first for m-TMXDI, then for the three source substances HDI, MDI, and TDI.
- 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

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

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 m-TMXDI

During the literature search performed for this dossier, only one report addressing potential RS in humans by m-TMXDI was identified. Grammer and co-workers (1993) reported an evaluation of 96 workers from facilities manufacturing or using m-TMXDI. While ca. 40 % of the workers reported to have experienced irritation of the upper respiratory tract and/or the eyes, no workers with new asthma or other severe respiratory symptoms were identified. Two workers reported exacerbation of a previously existing asthmatic disease. Serological assessments showed m-TMXDI-specific IgE antibodies in one and m-TMXDI-specific IgG antibodies in eight workers. Overall, 12 % of the workers exposed to estimated maximum concentrations of 0.4 to 10.2 ppb tested positive for m-TMXDI-specific antibodies. This report, however, shows a number of significant limitations:

- symptoms were only self-reported and respiratory function tests were not performed,
- no follow-up investigation was performed on those workers tested positive for specific antibodies,
- no information was provided on the possible origin of asthma (e.g. previous professional contact with isocyanates?) in the two reported exacerbation cases,
- the estimated exposure levels were quite low (with true exposure being unknown),
- no information was provided on whether all of the workers on survey had worked in the factory over the whole period of the study (1984-1988), and
- no information was provided on whether during this period workers had left the factory, in particular after the early phase of factory setup (identified by the authors as a phase of potentially higher exposure) and, if so, whether these workers had shown symptoms of respiratory disease.

In particular the last point introduces an unknown, but potentially significant amount of bias.

In summary, since evidence of immunological reactions in a number of workers was shown, these results are not suitable to demonstrate the absence of a potential of m-TMXDI to cause RS in humans. Contrary to the view of the authors, they are also not suitable to rank m-TMXDI as a respiratory sensitiser of "low" or "lower" potency than other diisocyanates (Grammer et al., 1993).

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 2 (case reports) and Tables 3-8 (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 m-TMXDI 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 3-8. 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 m-TMXDI has been evaluated and filtered for relevant studies (the complete list of studies is available in Table 9 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 vapor),
- 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. For studies with m-TMXDI, however, all studies meeting the above criteria (inhalation route, reliability) are described below, regardless of whether an effect was observed or not.

Finally, studies using agents other than m-TMXDI or the three source substances (as per Table 7) 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 the DS, 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 39 experiments from 21 study reports, performed in guinea pigs, mice, and rats qualified for further evaluation. Table 8 provides an overview of the number of studies and their distribution over the different substances and rodent species.

Table 8: Overview of the number of available animal experiments per substance and species

Diisocyanate	Species			Total
	Guinea pigs	Mice	Rats	
m-TMXDI	3	-	-	3
HDI	-	3	-	3
MDI	6	-	6	12
TDI	14	7	-	21
Total	23	10	6	39

10.6.5.1 Animal data for the target substance m-TMXDI

For m-TMXDI, three potentially relevant animal studies/experiments with inhalation exposure were identified, which are summarised in Table 9 (Bio-Research Laboratories, 1984a; Bio-Research Laboratories, 1984b; Union Carbide, 1988). For all of the studies only IUCLID summaries submitted by the lead registrant were available.

Table 9: Summary table of animal studies on sensitisation after induction via inhalation with m-TMXDI

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, vehicle	Study protocol	Results	Reference
Not applicable Range-finding study GLP: no data Reliability 3 (not reliable): Only IUCLID summary available, inconsistencies in reporting the treatment of control groups, spectrum of effect parameters assessed did not include more sophisticated respiratory function tests (only respiratory rate was measured). Reportedly, antibody analysis was performed, but results were not provided in the summary.	Guinea-pig, English Smooth-Haired, F, 8/group	Induction: m-TMXDI, no vehicle Challenge: m-TMXDI-Guinea-pig serum albumin (GPSA) conjugate in GPSA	Induction (days 1-5): 3 h/d with an atmospheric concentration of 24 µg/L by inhalation Challenge (day 8): Intradermal injection 25 µL of 0.0225 or 0.225 % solution of m-TMXDI-GPSA Skin reactions were evaluated after 24 and 48 h Terminal sacrifice on day 10	*"No evidence of increase in respiratory rate was seen in controls. Labored respiration and nasal oral discharge occurred in treated groups during the induction exposures. Slightly reduced body weights were observed. Lung weights and the histological appearance of the lungs of animals remained comparable with those of the controls. Slightly prominent bronchial and cervical lymph nodes were apparent macroscopically. Intradermal challenges with test material elicited clear erythemal response compared with controls."*	(Bio-Research Laboratories, 1984a)
Not applicable GLP: claimed Reliability 3 (not reliable): Only IUCLID summary available Only one treatment group, spectrum of effect parameters assessed did not include more sophisticated respiratory function tests (only respiratory rate was measured). Reportedly, antibody analysis was performed, but results were not provided in the summary.	Guinea-pig, English Smooth-Haired, F, 12/group	Induction: m-TMXDI, no vehicle Challenge: m-TMXDI-Guinea-pig serum albumin (GPSA) conjugate in GPSA	Induction (inhalation): 5 x 3 h/d to 36 µg/L air Rest period of 10-14 d Inhalation challenge: 20 min exposures to 15-20 µg/L m-TMXDI-GPSA/L air on days 22, 23, and 26 Intradermal challenge: Injection of 100 µL of 0.0333 % solution of m-TMXDI-GPSA on day 24 Skin reactions were evaluated after 6, 22 and 46 h Terminal sacrifice on day 26	*„Lethargy as well as nasal and oral discharge were observed in treated groups during the induction exposures. Body weights, lung weights and the histological appearance of the lungs of animals remained comparable with those of the controls. Intradermal and respiratory challenges with test material did not elicit any response indicative of sensitization."*	(Bio-Research Laboratories, 1984b)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, vehicle	Study protocol	Results	Reference
Not applicable GLP: claimed Reliability 2 (reliable with restrictions): Spectrum of effect parameters assessed did not include more sophisticated respiratory function tests (only respiratory rate was measured). High mortality (4/12 animals on days 2 (2 animals), 19 and 25)	Guinea pig, Hartley, F, 12	Induction: m-TMXDI, no vehicle Challenge: m-TMXDI-Guinea-pig serum albumin (GPSA) conjugate in GPSA	Induction (inhalation): 3 h/d to 30 µg/L TMXDI aerosol for 5 d Challenge (inhalation) on days 22, 23 and 26: 20 min to air followed by 20 min to 15-20 µg/L GPSA; recovery period of 30 min followed by 20 min to TMXDI-GPSA Day of sacrifice on day 26	*"Clinical signs of periocular, perioral, and perinasal wetness were observed along with respiratory difficulties and diminished motor activity in TMXDI-exposed animals. Four of the twelve TMXDI-exposed animals died during the study. Histopathologic examination of the lungs of TMXDI-exposed animals surviving until the end of the study showed a greater incidence and degree of alveolar histiocytosis than the lungs of control animals.* *A pulmonary hypersensitivity response was defined as a sustained increase (> 36 %) over the mean pre-exposure rate. An immediate pulmonary hypersensitivity response measured in terms of increased respiratory rates was not elicited from any of the guinea pigs upon inhalation challenge.* ***Low, but positive antibody titers for TMXDI were observed in exposed guinea pigs****."*	(Union Carbide, 1988)

All in all, beyond a weak indication of possible antibody formation of unknown type, none of these studies can reliably contribute to the identification of m-TMXDI as a respiratory sensitiser. By no means can they be used to prove the absence of RS potential in humans. As mentioned before, due to the lack of a standardised animal test design with regulatory acceptance, negative findings from such experiments cannot be used to exclude the need for classification and labelling for RS.

In addition, two of these studies (Bio-Research Laboratories, 1984a; Bio-Research Laboratories, 1984b) had quality issues in design and reporting (cf. column "Remarks" in Table 9 above) and assessed only a limited spectrum of effect parameters.

The only other available study followed a similar design (3 h/d exposures on five consecutive days, followed by three inhalation challenges with m-TMXDI-GPSA ca. two weeks later) and used a similar induction concentration. Consequently, also in this study no effects on the respiratory rate were observed. However, the author of the summary noted *"low, but positive antibody titers for TMXDI were observed in exposed guinea pigs"* but did not further specify the nature of these antibodies (Union Carbide, 1988).

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

Table 10 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 10: 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 15 for abbreviations)

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		

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.

[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.

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

Although providing some evidence of specific antibody formation, human data for m-TMXDI are by themselves not sufficient for classifying this substance as a respiratory sensitiser. 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 the available data for m-TMXDI give some indication of substance-related antibody formation, but are otherwise not sufficient on its own to justify classification for RS. 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.

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 m-TMXDI, only one study in humans is available which, however, is not adequate for classification and labelling, 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 category source substances HDI, MDI, and TDI evaluated for this dossier report positive bronchial provocation tests with these substances. 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 m-TMXDI as Resp. Sens. 1 in accordance with the CLP regulation.

10.6.7.2 Animal data

One study with m-TMXDI, which, however is considered to be of limited reliability, documented the production of specific antibodies following the exposure of guinea pigs to m-TMXDI by inhalation. In addition, 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 m-TMXDI,
- supplementary information on m-TMXDI, and
- the potential of m-TMXDI to cause skin sensitisation (cf. section 10.7 below),

the DS concludes that m-TMXDI 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.

10.7 Skin sensitisation

To the knowledge of the DS, no studies of the skin sensitising potential of m-TMXDI in humans are available. However, skin sensitisation test data in animals (BRC, 1981), summarised in the tables below as well as in Annex I to this dossier, are available for m-TMXDI, 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 11: Summary table of animal studies on skin sensitisation

Method, guideline, deviations	Species, strain, sex, no/group	Test substance, vehicle	Study protocol	Results	Reference
Similar to OECD 406 (Buehler Test), GLP claimed Reliability 2 (reliable with restrictions): Only study summary available, only 10 animals per group , non-occlusive exposure, only one induction application, challenge earlier than days 27-29, also irritant doses used for challenge	Guinea pig, Hartley, Primary Skin Irritation: 5 animals/dose. Induction: 10 animals/dose (two sites per animal) Challenge: 10 animals/dose	m-TMXDI in olive oil	Prior to the induction application, the primary irritation potential was determined. <u>Induction</u> 25 µL of a 0.36 mol/L (88 g/L or 9 %) solution of the test material in olive oil was applied epicutaneoulsy (non-occlusive) on day 1. <u>Challenge and rechallenge</u> 0, 0.10, 0.05, 0.025, 0.0125 and 0.00625 % applied epicutaneously (non-occlusive) 5 and 14 d after single induction application. Positive Control: IPDI	Positive, with up to 100 % of the test group sensitised depending on concentration (cf. Table 12)	(BRC, 1981)

Table 12: Results from a study on skin sensitisation with m-TMXDI (BRC, 1981)

Reading	Challenge dose level	No. with reactions (%)
1 (24 h post-challenge)	0.1 and 0.05 %	10 (100)*
	0.025 %	7 (70)*
	0.0125 %	9 (90)
	0.00625 %	5 (50)
2 (48 h post-challenge)	0.1, 0.05, 0.025 and 0.0125 %	10 (100)
	0.00625 %	7 (70)
Re-challenge (24 h post-challenge)	0.1, 0.05, 0.025, 0.0125 and 0.00625 %	0 (0)
Re-challenge (48 h post-challenge)	0.1, 0.05, 0.025, 0.0125 and 0.00625 %	0 (0)

* According to the summary in the registration dossier, these doses were slightly irritant (grade 1 erythema) in 2/5 females and irritant (grade 2 erythema) in 1/5 males tested during the primary skin irritation phase. Apparently, the figures given here refer to the number of animals with erythema of a higher grade than observed in the primary skin irritation phase; however, individual scores are not given in the summary.

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

In a skin sensitisation test with m-TMXDI similar to the Buehler protocol (BRC, 1981), between 50 and 100 % of the treated animals showed a positive response both 24 and 48 h post-challenge, depending on the challenge concentration (cf. Table 12 above). For all of the four highest challenge doses (0.0125-0.1 %) responses were 70 % or greater (but cf. footnote to Table 12). Upon re-challenge 24 or 48 h post-challenge, no positive reactions were reported. The reason for this is unclear, but it is noted that also the positive control (IPDI) gave only lower or no positive results upon re-challenge which might indicate experimental problems at the re-challenge step. In addition, the test protocol used showed some deviations from the Buehler test method as laid out in OECD TG 406. In the view of the DS, those deviations (less animals used, only one instead of three induction exposures, non-occlusive exposure, early challenge) all tend to decrease the sensitivity of the test and a negative test result would not have been acceptable in this case. However, since clear positive results were obtained, the DS rates this study as “reliable with restrictions” or Klimisch code 2.

Table 13: Comparison of experimental results confirming the skin sensitisation potential of m-TMXDI in animals with the respective criteria of the CLP regulation and CLP guidance

Criteria acc. to Table 3.4.3 and Table 3.4.4 of the CLP regulation and Table 3.8 of the CLP guidance		Reference(s)	Sensitisation rate (%)/Topical induction dose (%)	Resulting Classification
Skin Sens. 1A, Extreme	≥ 60 % responding at ≤ 0.2 % topical induction dose	(BRC, 1981)	≤ 100/9	**Skin Sens. 1A** **Strong sensitiser**
Skin Sens. 1A, Strong	≥ 15 - < 60 % responding at ≤ 0.2 % topical induction dose or ≥ 60 % responding at > 0.2 - ≤ 20 % topical induction dose			
Skin Sens. 1B, Moderate	> 15 - < 60 % responding at > 0.2 - ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose			

10.7.1 Comparison with the CLP criteria

According to the criteria given in Table 3.4.3 of the CLP regulation, skin sensitisers fall into category 1A based on the results from a Buehler test, if 60 % or more of the animals show a positive response at a topical induction concentration of > 0.2 to ≤ 20 %. This criterion was fulfilled for four of the five challenge doses tested (and consistently so at both the first and second reading).

10.7.2 Conclusion on classification and labelling for skin sensitisation

Based on the test results in guinea pigs, m-TMXDI should be classified as Skin Sens. 1A (hazard statement H317: May cause an allergic skin reaction).

10.8 Germ cell mutagenicity

Not relevant for this dossier

10.9 Carcinogenicity

Not relevant for this dossier

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-TMXDI:

"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.'

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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-Response-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: Isophorone-diisocyanate

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'-Methylene-diphenyldiisocyanate

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

TOE: Toepad inoculation

TOP: Topical

TWA: Time-weighted average

VP: Vapour

WB: Whole-body