# Committee for Risk Assessment

# RAC

Annex 1

**Background document**

to the Opinion proposing harmonised classification and labelling at EU level of

# 1,3-bis(isocyanatomethyl)benzene

## EC Number: 222-852-4
## CAS Number: 3634-83-1

CLH-O-0000006846-62-01/F

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

## Adopted

## 17 September 2020

# CLH report

# Proposal for Harmonised Classification and Labelling

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

# International Chemical Identification:

## *Bis(isocyanatomethyl)benzene;*

## *[m-XDI]*

**EC Number:** **222-852-4**

**CAS Number:** **3634-83-1**

**Index Number:** **n.a.**

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**Date:** **July 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,3-BIS (ISOCYANATOMETHYL)BENZENE

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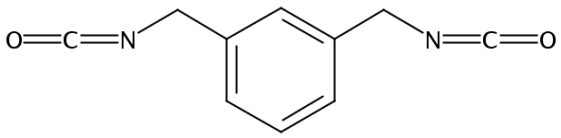
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,3-BIS (ISOCYANATOMETHYL)BENZENE

# 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(isocyanatomethyl)benzene |
| **Other names (usual name, trade name, abbreviation)** | m-Xylene diisocyanate<br>m-XDI<br>XDI |
| **ISO common name (if available and appropriate)** | - |
| **EC number (if available and appropriate)** | 222-852-4 |
| **EC name (if available and appropriate)** | 1,3-Bis(isocyanatomethyl)benzene |
| **CAS number (if available)** | 3634-83-1 |
| **Other identity code (if available)** | - |
| **Molecular formula** | $C_{10}H_8N_2O_2$ |
| **Structural formula** |  |
| **SMILES notation (if available)** | O=C=NCC1=CC(=CC=C1)CN=C=O |
| **Molecular weight or molecular weight range** | 188.18 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(isocyanatome-thyl)benzene<br>EC No. 222-852-4<br>CAS No. 3634-83-1 | 80-100 | - | Flam Liq. 3, Acute Tox. 1/2 (H330), Acute Tox. 3 (H331), Skin Corr. 1B (H314), Skin Irrit. 2 (H315), Eye Dam. 1 (H318), Eye Irrit.2 (H319), Resp. Sens. 1 (H334), Skin Sens. 1A/1 (H317), STOT SE 1 (H370, respiratory tract, inhalation), STOT SE 3 (H335, inhalation), STOT RE 1 (H372, respiratory tract, inhalation), Aquatic Chronic 3 (H412) |

# 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-XDI

| | 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(isocyanatomethyl)benzene; [*m*-XDI] | 222-852-4 | 3634-83-1 | Resp. Sens. 1<br>Skin Sens. 1A | H334<br>H317 | GHS08<br>Dgr | H334<br>H317 | | Skin Sens. 1A; H317: C ≥ 0,001 % | |
| 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.

| **RAC general comment** |
| --- |
| 1,3-bis(isocyanatomethyl)benzene (m-XDI) has no current entry in Annex VI to the CLP Regulation. The substance is used for manufacture of plastic products and is self-classified as Resp. Sens. 1, and/or Skin Sens. 1 or Skin Sens. 1A.<br><br>The Dossier Submitter (DS) mentioned that according to Article 36 of the CLP regulation, respiratory sensitisation is an endpoint for which Harmonised Classification and Labelling (CLH) is warranted, and skin sensitisation is closely linked to respiratory sensitisation. Namely, all currently known low molecular weight chemical respiratory sensitisers are also skin sensitisers.<br><br>The CLH report has been created based on the data submitted by the lead registrant in the REACH registration dossier for m-XDI, and further relevant data were retrieved as part of a general literature search in the context of the restriction proposal for diisocyanates recently submitted to ECHA by the DS. |

## 5 IDENTIFIED USES

A summary of the information available on ECHA's public website (accessed 2017-12-14) 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.

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

### 5.4 Widespread use by professional workers

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

### 5.5 Formulation or re-packing

This substance is used in the following products: polymers. This substance is used in the following activities or processes at workplace: closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, mixing in open batch processes, transfer of chemicals at dedicated facilities, transfer of substance into small containers and laboratory work. Release to the environment of this substance can occur from industrial use: formulation of mixtures.

### 5.6 Uses at industrial sites

This substance is used in the following products: polymers, adhesives and sealants and coating products. This substance is used in the following areas: formulation of mixtures and/or re-packaging. This substance is used for the manufacture of: plastic products. This substance is used in the following activities or processes at workplace: closed batch processing in synthesis or formulation, closed processes with no likelihood of exposure, transfer of chemicals at dedicated facilities, laboratory work, closed, continuous processes with occasional controlled exposure, transfer of substance into small containers and batch processing in synthesis or formulation with opportunity for exposure. Release to the environment of this substance can occur from industrial use: as an intermediate step in further manufacturing of another substance (use of intermediates).

### 5.7 Manufacture

This substance is used in the following activities or processes at workplace: closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, transfer of chemicals at dedicated facilities, transfer of substance into small containers and laboratory work. Release to the environment of this substance can occur from industrial use: manufacturing of the substance.

## 6 DATA SOURCES

This report has been created based on the data submitted by the lead registrant in the REACH registration dossier for m-XDI. In addition, further relevant data on m-XDI 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 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** | Freezing temperature:<br>-7 °C (266 K) | Experimental result<br>[OECD Guideline 102 (Melting point/Melting Range): thermal analysis (differential scanning calorimetry (DSC)] |
| **Boiling point** | No boiling point; decomposition starting at 175 °C (448K) | Experimental result<br>[OECD Guideline 103 (Boiling point/boiling range): differential scanning |

| Property | Value | Comment (e.g. measured or estimated) |
|---|---|---|
| | | calorimetry] |
| **Relative density** | 1.20 (at 20 °C) | Experimental result<br>[OECD Guideline 109 (Density of Liquids and Solids): pycnometer method (volume pycnometer)] |
| **Vapour pressure** | 0.0206 Pa (room temperature) | Experimental result<br>[OECD Guideline 104 (Vapour Pressure Curve): gas saturation method] |
| **Vapour pressure, ctd.** | Calculated vapour pressure of the hydrolysis product XDA ((3-(aminomethyl)phenyl)methanamine): 1.95 Pa (at 25°C) | Calculated value<br>[QSAR (MPBVP program, version 1.43; Modified Grain Method)] |
| **Surface tension** | N.a. (substance reacts with water) | - |
| **Water solubility** | N.a. (substance reacts with water) | - |
| | Calculated water solubility: 106.27 mg/L (at 25°C, pH 7.0) | Calculated value<br>[QSAR (WATERNT program (version 1.01), part of EPI Suite)] |
| | Calculated water solubility of the hydrolysis product XDA ((3-(aminomethyl)phenyl)methanamine): 656.45 g/L (at 25°C, pH 7.0) | |
| **Partition coefficient n-octanol/water** | Calculated logKow: 3.00 (at 25°C) | Calculated value<br>[QSAR (KOWWIN (version 1.67), part of EPI Suite)] |
| | Calculated logKow: 3.22 (at 25°C) | Calculated value<br>[QSAR (ACD PhysChem, version 12.01)] |
| | Calculated logKow: 2.99 (at 25°C) | Calculated value<br>[QSAR (ACD ADME Suite (version 5.07))] |
| | Calculated logKow of the hydrolysis product XDA ((3-(aminomethyl)-phenyl)methanamine): 0.15 (at 25 °C) | Calculated value<br>[QSAR (KOWWIN (version 1.67), part of EPI Suite)] |
| **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: 6 mPa s (at 25 °C) | Experimental result<br>[JIS K 7117: Rotational viscometer (dynamic)] |

## 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-XDI are available. In the REACH 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-XDI

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| Property | Estimate by Registrant | DS Comment |
|---|---|---|
| **Hydrolysis and metabolism** | *"As XDI is a diisocyanate, it reacts with water. Isocyanates hydrolyse readily in water to yield a carbamic acid, which decarboxylates to produce $CO_2$ and an amine; the latter can immediately react with more isocyanate to yield a disubstituted urea. The hydrolysis rate increases with electron-withdrawing substituents. Steric hindrance also influences hydrolysis rate. Even at low pH, a hydrolysis half-life < 10 minutes (25 °C) has been found for isocyanates."* | No hydrolysis study has been submitted with the lead registration dossier. The registrant has justified this with the statement "As the substance reacts with water the hydrolysis study was not performed. Based on experience with isocyanates, the half-life of XDI in water is considered to be significantly less than 12 hours."<br>The DS notes that there are no data supporting this statement and that it would have been exactly the purpose of a hydrolysis study to provide such data. Moreover the DS further notes that for a close structural analogue, m-TMXDI (CAS 2778-42-9), a hydrolysis study according to OECD TG 111 is available (only as an IUCLID summary in the respective lead registration dossier). |

| Property | Estimate by Registrant | DS Comment |
|---|---|---|
| **Hydrolysis and metabolism, ctd.** | *"Physico-chemical characteristics of m-XDI requiring studies in aqueous surroundings (as water solubility, octanol/water partition coefficient and surface tension) can therefore not be determined on m-XDI itself.*<br><br>*After absorption, m-XDI will react with water as stated above, yielding carbamic acid, $CO_2$, amine and disubstituted urea."* | Depending on pH and temperature, the reported rate constants and estimated half-lives were as follows (Wooley and Mulley, 2003):<br>- 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",<br>- pH 4, 25 ± 0.5 °C: 1.692 $h^{-1}$/0.410 h,<br>- pH 7, 25 ± 0.5 °C: 1.9044 $h^{-1}$/0.364 h,<br>- pH 9, 37 ± 0.5 °C: 2.0664 $h^{-1}$/0.336 h.<br>These figures indicate that 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 about 40-50 min, 12.5 % after about 80-90 min etc.). In the view of the DS this provides a sufficiently large time window for the initial steps of sensitisation to take place. In addition it is also noted that reactions of m-XDI with proteins to form a protein-hapten complex would compete with hydrolysis due to moisture on the skin or within the respiratory tract, and thus the fraction of m-XDI effectively available for sensitisation can be expected to be even greater than suggested by the above figures.<br>Finally, 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** | *"Moderate log P values (between -1 and 4) are favourable for absorption directly across the respiratory tract epithelium by passive diffusion. The low vapour pressure of the substance (0.0206 Pa) indicates that the substance will not be available for inhalation as vapour. The relatively low water solubility is favourable for penetration to the lower respiratory tract. Based on these physico-chemical properties of XDI, for risk assessment purposes the inhalation absorption is set at 100 %.*<br><br>*The results of the toxicity studies do not provide reasons to deviate from this proposed inhalation absorption percentage. m-XDI, being a liquid, has the potential to be dermally absorbed. The moderate log Pow of 3.07 is also indicative of dermal absorption. The criteria for 10 % dermal absorption as given in the REACH Guidance on Information Requirements and Chemical Safety Assessment (MW > 500 and log P is outside of the range -1 to 4) is not met, and therefore 100 % dermal absorption of m-XDI should be considered for risk assessment purposes."* | The statements of the registrant correctly reflect the content of the guidance which 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."<br>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. |

| Property | Estimate by Registrant | DS Comment |
|---|---|---|
| **Absorption via inhalation and the dermal route, ctd.** | *"Although it is generally accepted that dermal absorption is not higher compared to oral absorption, 100 % dermal absorption should be considered for risk assessment purposes as m-XDI has skin irritating properties and damage to the skin surface may enhance penetration."* | Knowledge about the systemic distribution (and eventual elimination) is therefore not needed for deciding qualitatively on the sensitisation potential of the diisocyanates. |
| **Bioaccumulation** | *"XDI does not have the potential to bioaccumulate in aquatic species due to its hydrolytical instability."* | The DS notes that the lead registrant does not provide data to support his assessment. However, for the close structural analogue m-TMXDI, 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-XDI is unlikely to possess a potential for bioaccumulation. |
| **Excretion** | *"Because of the relatively small molecular weight of m-XDI, the hydrolysis products and/or metabolites will either be excreted via the bile or the urine."* | The DS notes that since all non-bioaccumulative chemicals entering systemic circulation are either excreted via bile or urine (unless they are exhaled), this statement does not contain any significant m-XDI-specific information. Moreover, again no data are provided in its support. On the other hand, excretion is not relevant for this dossier which focuses on sensitisation hazard. |

# 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-XDI, the substance addressed by this dossier, data with respect to RS are scarce. For m-XDI, specifically, no human or animal data related to RS were identified by the DS.

Article 9 of the CLP regulation specifies how the hazard information is evaluated to decide on classification. The strategy followed in this dossier is therefore characterised by a category approach by means of which the knowledge about the RS potential of the three most commonly used diisocyanates HDI, MDI, and TDI is read across to m-XDI. 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.

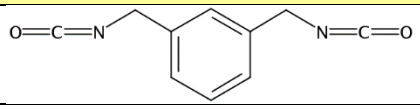
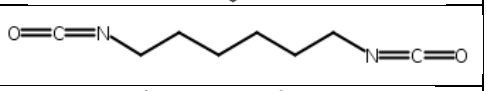
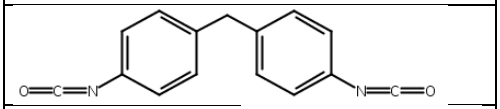
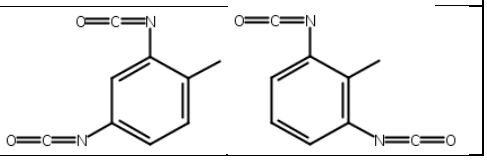
#### 10.6.2.2 AE C.1 Substance characterisation

The identity of the target substance m-XDI has been characterised above. Table 7 below provides information on the identity and harmonised classification of the target substance as well as the category source substances HDI, MDI, and TDI.

#### 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.

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

| EC Name; trivial name used in this report | EC No. CAS no. | CLH for sensitisation (Annex VI to CLP) | Structure |
|---|---|---|---|
| 1,3-Bis(isocyanatome-thyl)benzene m-XDI | 222-852-4<br>3634-83-1 | - |  |
| Hexamethylene diisocyanate; HDI | 212-485-8<br>822-06-0 | Resp. Sens. 1<br>Skin Sens. 1 |  |
| 4,4'-Methylenediphenyl diisocyanate; MDI[$] | 202-966-0<br>101-68-8 | |  |
| m-Tolylidene diisocyanate (80/20 mixture of 2,4-TDI and 2,6-TDI isomers); TDI[$] | 247-722-4<br>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.

[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.

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

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

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

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

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

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

### 10.6.2.7 AE 6.1 Compounds the test organism is exposed to

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

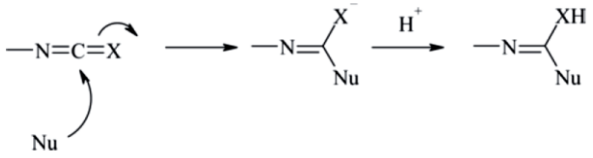



Figure 1: Acylation reaction for isocyanates (X = oxygen). Reproduced from (Enoch et al., 2011)

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

The potential reactivity of the diisocyanate source substances given in Table 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 are 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-XDI, HDI, MDI, and TDI. Notably, numerous studies demonstrate the ability of diisocyanates to cause symptoms of RS also after dermal exposure (cf. the restriction report for diisocyanates recently submitted by the German MSCA[3]), however, since the definition from the CLP regulation cited in section 10.6.1 clearly asks for inhalation exposure, only studies along this route were evaluated for the current dossier.
- Evaluate and present the relevant human data for the three source substances HDI, MDI, and TDI (no relevant data were identified for m-XDI).
- Filter animal data for relevance according to predefined criteria (cf. section 10.6.5).
- Evaluate and present the relevant animal data for the three source substances HDI, MDI, and TDI (no relevant data were identified for m-XDI).
- Summarise, compare to the CLP criteria and conclude on a possible potential for RS.

### 10.6.4 Human data

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

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

Both immediate (seconds to minutes) and late-onset (up to several hours) hypersensitivity reactions may be present in patients with diisocyanate-induced asthma, with the prevalence of late responses being as high as 70 % (Niimi et al., 1996). The delay between onset of (low-level) exposure at work and the manifestation of the asthmatic symptoms, which may be as long as several years after the start of exposure, is of particular concern. In addition, patients often develop persistent bronchial hyperresponsiveness (BHR; often also the more general term "airway hyperresponsiveness/hyperreagibility (AHR)" is used interchangeably) to non-

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

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-XDI

No relevant data for m-XDI were identified during the literature search performed for this dossier.

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

More than 100 case reports and epidemiological studies have been evaluated. An overview of this evaluation is provided in Annex I, Table 1 (case reports) and Tables 2-7 (epidemiological studies). The case reports provide overwhelming proof that humans exposed to the source substances HDI, MDI, and/or TDI may suffer from a broad spectrum of respiratory effects including asthma and pathological changes of the airways. Also a number of fatal cases have been reported, albeit not in recent years. While during the early stages of the development of the disease, respiratory symptoms may eventually be reversed upon removal from exposure, an irreversible remodelling of the airways will eventually take place when exposure is continued. On the other hand these case reports do not allow for an assessment of the frequency of occurrence of respiratory sensitisation to m-XDI 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 individuals is reported). They are therefore not suited for sub-categorisation. In addition, no harmonised approach for sub-categorising respiratory sensitisers is available yet.

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

### 10.6.5 Animal data

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

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

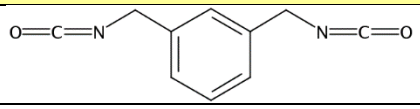
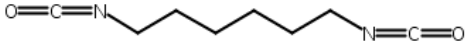
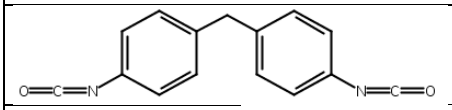
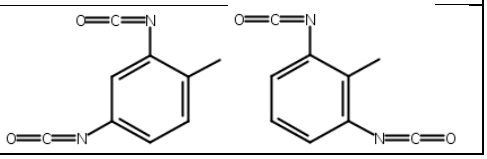
From this wording the DS concludes that (test substance-specific) changes in immunological parameters as well as specific pulmonary responses may be important indicators of RS, whereas the absence of such effects in animals cannot serve as a proof of the absence of RS potential in humans. With respect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

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

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

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

Animal study designs for respiratory sensitisation have been manifold, involving a variety of species, protocols, and target endpoints, and a standardised protocol with regulatory acceptance is still missing. Therefore a negative result from an animal experiment on RS is not suitable to exclude the need for classification and labelling. Consequently, for the read-across assessment the evaluation concentrated on data providing a positive indication of respiratory sensitisation, therefore for HDI, MDI, and TDI only studies reporting the presence of one or more relevant effects were selected for further processing. Where several experiments were reported in one study report, only those with effects were processed further. Finally, studies using agents other than m-XDI or the three source substances (as per Table 7: Overview of target and category source substances used for read-across to m-XDI

| **EC Name; trivial name used in this report** | **EC No. CAS no.** | **CLH for sensitisation (Annex VI to CLP)** | **Structure** |
|---|---|---|---|
| 1,3-Bis(isocyanatomethyl)benzene m-XDI | 222-852-4 3634-83-1 | - |  |
| 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.

) 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 36 experiments from 18 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-XDI** | - | - | - | - |
| **HDI** | - | 3 | - | 3 |
| **MDI** | 6 | - | 6 | 12 |
| **TDI** | 14 | 7 | - | 21 |
| **Total** | 20 | 10 | 6 | 36 |

### 10.6.5.1 Animal data for the target substance m-XDI

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

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

Table 9 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 9: 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 | SS | |
| | | | IDE | TDI-GPSA | | | 8 | | | | | |
| | | | INH | TDI-GPSA/ TMI-GPSA | | | 12 | | | | RF | |
| DH | F | TDI | INH | TDI-GPSA | AE | NO | 10 | 5 | 3 | 5 | AB/RF | (Botham et al., 1988) |
| DH | F | MDI | - | - | VP | NO | 5 | 5 | 3 | 21 | AB | (Dearman and Botham, 1990) |
| | | | IPE | MDI-GPSA | | | | | | 22 | | |
| Hartley | F | TDI | INH | TDI | VP | WB | 7 | 5 | 3 | 21 | AB/IF/RF | (Huang et al., 1993a) |
| Hartley | F | TDI | INH | TDI | VP | WB | 6 | 5 | 3 | 26 | AB/RF | (Aoyama et al., 1994) |
| Hartley | ? | MDI | INH | MDI | AE | NO | ≥ 8 | 1 | 0.25 | 21/ 22 | RF | (Pauluhn, 1994) |
| | | | | MDI-GPSA | | | | | | | | |
| | | TDI | | TDI | VP | | | | | | | |
| | | | | TDI-GPSA | | | | | | | | |
| DH | F | MDI | INH | MDI | AE | NO | 16 | 5 | 3 | 18 | AB | (Rattray et al., 1994) |
| ? | ? | MDI | INH | MDI | AE | NO | 16 | 1 | 0.25 | 21/ 28 | AB/RF | IUCL: (Bayer, 1995) |
| DH | F | TDI | - | - | VP | WB | 20 | 1 | 48 | 3 | RF | (Gagnaire et al., 1996) |
| | | | | | | | | | 168 | 8 | | |
| DH | F | TDI | - | - | VP | WB | 10 | 1 | 1344 | 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 | | |

10.6.5.2.1 Guinea pigs

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

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

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

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

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

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

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

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

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

10.6.5.2.2 Mice

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

Ban and co-workers induced sensitisation in female BALB/c mice by 4 h-exposure via whole-body inhalation to 3 ppm TDI on three consecutive days[4]. Challenge was either performed by two single 4 h challenges with 0.3 ppm TDI 7 or 12 days after the end of induction or by a single 4 h inhalation challenge with 2 ppm TDI 14 days after the end of induction, followed by a 1 d tracheal instillation with 50 µg TDI-HAS conjugate/animal one week later. The authors reported increases in a number of inflammation markers including cytokines (with some variability between the two designs) as well as a statistically significant rise of total IgE antibody levels (Ban et al., 2006).

Arts and colleagues used a "respiratory local lymph node assay", i.e. a study protocol in which male Balb/c mice were first exposed once per day on three consecutive days to HDI or TDI by inhalation, followed by an evaluation of the proliferation of the draining mandibular lymph nodes three days later. Both diisocyanates caused marked proliferation with the stimulation index exceeding a value of 3 at all inhalation concentrations applied (Arts et al., 2008; de Jong et al., 2009).

10.6.5.2.3 Rats

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

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

#### 10.6.6.1 Human data

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

#### 10.6.6.2 Animal data

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

[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.

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-XDI, no study in humans is available, a category approach is used for classification in accordance with CLP Article 5 1. (2) referring to REACH Annex XI, section 1. Numerous case reports and epidemiological studies with the source substances HDI, MDI, and TDI evaluated for this dossier report positive bronchial provocation tests with these substances. 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-XDI as Resp. Sens. 1 in accordance with the CLP regulation.

#### 10.6.7.2 Animal data

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

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

### 10.6.8 Conclusion on classification and labelling for respiratory sensitisation

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

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

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

## RAC evaluation of respiratory sensitisation

### Summary of the Dossier Submitter's proposal

The DS proposed to classify m-XDI as Resp. Sens. 1 (H334). Currently, m-XDI does not have a harmonised classification. Its self-classification and labelling according to CLP is variously: Flam Liq. 3, Acute Tox. 1 or 2 (H330), Acute Tox. 3 (H331), Skin Corr. 1B (H314), Skin Irrit. 2 (H315), Eye Dam. 1 (H318), Eye Irrit. 2 (H319), Resp. Sens. 1 (H334), Skin Sens. 1A/1 (H317), STOT SE 1 (H370, respiratory tract, inhalation), STOT SE 3 (H335, inhalation), STOT RE 1 (H372, respiratory tract, inhalation), Aquatic Chronic 3 (H412).

There is no specific human or animal respiratory sensitisation (RS) data available for m-XDI. Therefore, the proposed harmonised classification was based on read across.

Only the three most commonly used source substances were used for read across from, as most of the published literature on diisocyanates is related to them: 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). They all have harmonised classifications as Resp. Sens. 1 (H334). The DS noted that several other diisocyanates also have a (self-)classification as respiratory sensitiser. The DS is not aware of any monomeric diisocyanates for which data convincingly show that the substance is not a respiratory (and skin) sensitiser. For HDI, MDI and TDI, there is an abundance of publicly available human and non-human data.

***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, and they all have harmonised classifications as Resp. Sens. 1 (H334).

According to the DS, the case reports provide overwhelming proof that humans exposed to the source substances 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 the respiratory symptoms may eventually be reversed upon removal of exposure, an irreversible remodelling of the airways will eventually take place if exposure is continued. On the other hand, these case reports do not enable an assessment of the frequency of occurrence of respiratory sensitisation in the human population because they feature only a small number of patients. It is also not known which fraction of all exposed individuals is affected and which fraction of the affected is reported. The case reports are therefore not suited for potency sub-categorisation. In addition, no harmonised approach for sub-categorising respiratory sensitisers is currently 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 aetiology of the disease. No study overcomes the problem that sensitive predictive markers for diisocyanate sensitisation are missing and cannot currently be assessed appropriately. In addition, dermal exposure and

inhalation peak exposure are both likely to contribute to the induction of sensitisation.

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% of patients. 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. Complete recovery of lung function may never be achieved and patients often develop persistent bronchial hyper-responsiveness (often also the more general term "airway hyper-responsiveness/hyper-reagibility" 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.

***Animal data for the read across source substances HDI, MDI and TDI***

There are no internationally recognised *in vivo* identification methods for respiratory sensitisation. Animal studies were considered by the DS to be relevant for the classification only if the induction route was truly via the inhalation route. Studies using other routes of induction or mixed routes were discarded. Furthermore, studies were considered unreliable and excluded from the assessment in case any of the following information was missing or incomplete: identity of the test substance, physical state of the test substance as applied (aerosol or vapour), 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 have been manifold, involving a variety of species, protocols and target endpoints, while a standardised protocol with regulatory acceptance is still missing. Therefore, while a negative result from an animal experiment on respiratory sensitisation is deemed as not sufficient to exclude the need for classification and labelling, the read across assessment concentrated on data providing a positive indication of respiratory sensitisation. HDI, MDI, and TDI studies reporting one or more relevant effects were selected for further processing, as outlined in the table below. 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, as 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 fluid (BALF). The observed respiratory symptoms (increased respiratory rate, effects on respiratory flow, laboured breathing etc.) resembled those seen in humans with asthma. In addition, skin sensitisation has been observed following induction via inhalation. However, the interdependencies and quantitative contributions of factors such as the species and strain used, concentration and total dose received upon induction, or the temporal pattern of dosing are still poorly understood.

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

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

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

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

***Read across from HDI, MDI and TDI to m-XDI***

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

The three source substances and the target substance m-XDI all share the structural feature of two isocyanate (-N=C=O) functional groups while the part of the molecular structure that links the two isocyanate groups are variable (see figure below).

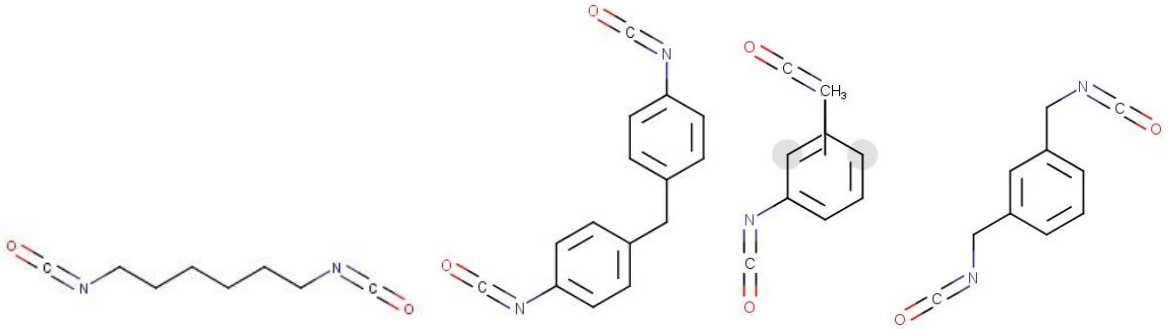


**Figure** The structures of HDI, MDI, TDI and m-XDI, respectively, from left to right.

The isocyanate (-N=C=O) functional group is a well-known structural alert for respiratory sensitisation, and therefore commonly used also in respiratory sensitisation prediction tools. It has been hypothesised and to a certain degree shown for respiratory sensitisers that, similarly to skin sensitisation, covalent binding of electrophiles to proteins in the lung marks a molecular initiating key event. For isocyanates, an acylation type reaction between electrophilic NCO chemical functional groups and nucleophilic protein moieties may occur, leading to protein adducts (Enoch *et al.*, 2011; Enoch *et al.*, 2009; Enoch *et al.*, 2014). Furthermore, it has been shown 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 high 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 isocyanate groups are of less importance, although they may have an impact on the physical-chemical and ADME properties of the compounds, and therefore influence their relative potencies (not addressed in the dossier).

## Comments received during consultation

Three MSCAs commented during the consultation. All of them supported the proposed classification as Resp. Sens. 1 (H334).

## Assessment and comparison with the classification criteria

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

For the source substances HDI, MDI and TDI, numerous case reports and epidemiological studies consistently demonstrate their 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 many other diisocyanates. Current mechanistic knowledge on the effects of diisocyanates shows that the effects depend

on the diisocyanate group while the rest of the molecular structure can vary considerably. In other words, the diisocyanate structure itself is widely accepted as an alert for respiratory sensitisation.

For m-XDI, the read across performed by the DS considered all of the assessment elements relevant for scenario 6 of the RAAF (Appendix F).

**Hazard category and sub-categories for respiratory sensitisers**

| Category | Criteria |
|---|---|
| Category 1 | Substances shall be classified as respiratory sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria: |

In addition to the CLP criteria for classification of a substance as a respiratory sensitiser, the CLP Regulation Annex I section 3.4.2.1.2.3 also 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"* (European Parliament and Council, 2008).

Regarding *in vivo* studies, section 10.6.5 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".*

Overall, RAC considers the weight of evidence assessment by the DS to be adequate. In addition, the Committee agrees with the justification for a category approach using read across (based on human and non-human data) from the known Cat. 1 respiratory sensitisers HDI, MDI and TDI to the target substance m-XDI. The read across by the DS is acceptable and performed according to RAAF. RAC also agrees that it is not possible to sub sub-categorise m-XDI into 1A or 1B, as no reliable data on the potency of either m-XDI 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 m-XDI.

## 10.7 Skin sensitisation

To the knowledge of the DS, no studies of the skin sensitising potential of m-XDI in humans are available. However, skin sensitisation test data in animals summarised in Table 10 below are available for m-XDI, 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 10: Summary table of the available animal studies on skin sensitisation for m-XDI

| Method, guideline, deviations | Species, strain, sex, no/group | Test substance, vehicle | Study protocol | Results | Reference |
|---|---|---|---|---|---|
| Similar to OECD TG 406 (GPMT), non-GLP<br><br>Reliability 3 (not reliable): Only IUCLID summary avail-able, insufficient reporting; ele-mentary information on experimental design is missing | Guinea pig, Dunkin-Hartley, male, 10/test group, 5/negative control group | m-XDI, Alembicol D | <u>Induction:</u><br>▪ Intradermal injection with 0.01 % v/v m-XDI in Alembicol D<br>▪ Topical induction: Undiluted m-XDI<br><br><u>Challenge:</u><br>Topical challenge with 20 % v/v m-XDI in acetone | Sensitisation is demonstrated in 9/10 animals, but unsuitable for classification and labelling due to insufficient reporting, how-ever, reported results are consistent with Skin Sens. 1 A | (Huntingdon, 1980) |
| Similar to OECD TG 406 (GPMT)/EU B.6<br><br>GLP claimed<br><br>Reliability 3 (unreliable): Only IUCLID summary available, no purity informa-tion provided, results are compromised by unclear degree of irritation seen in controls | Guinea pig, Dunkin-Hartley, female, 20/test group, 10/control group | m-XDI, Arachis oil BP | <u>Induction</u><br><br>*Intradermal*<br>Three injections, 0.1 mL each:<br>▪ Freund's Complete Adjuvant (FCA)/distilled water 1:1,<br>▪ 0.1 % w/v formulation of the test material in vehicle,<br>▪ 0.1 % w/v formulation of the test material in a 1:1 preparation of FCA plus vehicle.<br><br>*Topical (day 7)*<br>Filter paper containing 75 % m-XDI for 48 h, occlusive dressing<br><br><u>Challenge (day 21)</u><br>24 h occlusive topical challenge with 50 and 75 % m-XDI on filter paper. | 100 % sensiti-sation rate at both challenge doses of 50 and 75 %, but unsuitable for classification and labelling due to insufficient reporting.<br><br>However, repor-ted results are consistent with Skin Sens. 1 A | (Safepharm, 1992) |

| Method, guideline, deviations | Species, strain, sex, no/group | Test substance, vehicle | Study protocol | Results | Reference |
|---|---|---|---|---|---|
| OECD TG 406 (GPMT)/EU B.6<br><br>GLP claimed<br><br>Reliability 2 (reliable with restrictions): No purity information provided, only summary available | Guinea pig, Dunkin-Hartley, male, 10/test group, 5/control group | m-XDI, Alembicol D | Induction<br><br>*Intradermal*<br>Three injections, 0.1 mL each:<br>▪ FCA/distilled water 1:1,<br>▪ 0.01 % w/v formulation of the test material in vehicle,<br>▪ 0.01 % w/v formulation of the test material in a 1:1 preparation of FCA plus vehicle.<br><br>*Topical (day 7)*<br>Filter paper containing 100 % m-XDI for 48 h, occlusive dressing<br><br>Challenge (day 21)<br>▪ 24 h occlusive topical challenge with 15 and 7.5 % m-XDI on filter paper, occlusive dressing | 100 % sensitisation rate at both challenge doses of 15 and 7.5 %<br><br>Extreme skin sensitiser; Skin Sens. 1A | (Huntingdon, 1997) |
| Similar to OECD TG 406 (GPMT)/EU B.6<br><br>GLP claimed<br><br>Reliability 3 (unreliable): No purity information provided, only IUCLID summary available, results of first challenge not reported, results reported for re-challenge are further compromised by an unclear degree of irritation seen in controls and treatment groups, indications of poor handling of animals | Guinea pig, Dunkin-Hartley, male, 10/test group, 5/control group | m-XDI, Arachis oil BP | Induction<br><br>*Intradermal*<br>Three injections, 0.1 mL each:<br><br>▪ FCA/distilled water 1:1,<br>▪ 0.01 % w/v formulation of the test material in vehicle,<br>▪ 0.01 % w/v formulation of the test material in a 1:1 preparation of FCA plus water.<br><br>*Topical (day 7)*<br>Filter paper containing 100 % m-XDI for 48 h, occlusive dressing<br><br>Challenge (day 21)<br>24 h occlusive topical challenge with 100 and 75 % m-XDI on filter paper<br><br>Re-challenge (day 41)<br>24 h occlusive topical challenge with 50 and 25 % m-XDI on filter paper | 100 % sensitisation rate at both re-challenge doses of 50 and 25 %, but unsuitable for classification and labelling due to deviations and insufficient reporting.<br><br>In addition, one test group animal was found dead (day 8), another had to be euthanised (day 10); erythema could not be scored due to undisclosed “adverse effects” in the control group, following re-challenge, two animals suffered from physical damage after removal of dressing.<br><br>However, reported results are consistent with Skin Sens. 1 A | (Safepharm, 1998) |

In a skin sensitisation test in guinea pigs, 9/10 animals reportedly displayed symptoms of dermal contact hypersensitivity 24-72 h after challenge with 20 % v/v m-XDI in acetone after previous sensitisation to m-XDI by a) an intradermal injection of 0.01 % v/v m-XDI in Alembicol D and b) a topical application of neat m-XDI. The lead registrant's summary of this study lacks elementary information on the experimental design and therefore, while indicating a potential of m-XDI to cause skin sensitisation in guinea pigs, this study cannot be used for classification and labelling (Huntingdon, 1980).

In a guinea pig maximisation test (GPMT) similar to OECD TG 406/EU B.6, m-XDI reportedly produced a sensitisation rate of 100 % with an intradermal induction dose of 0.1 % and challenge doses of 75 and 50 %. However, the study summary available from the registration dossier also reported erythema in both the challenged test and control groups which could be indicative of irritation. As erythema scores were not reported, the results of this summary are considered unreliable and cannot be used for classification and labelling. It is however noted that the reported results are consistent with m-XDI being an extreme sensitiser (Safepharm, 1992).

In an OECD TG 406/EU B.6-conform guinea pig maximisation test (GPMT), m-XDI produced a sensitisation rate of 100 % with an intradermal induction dose of 0.01 % and challenge doses of 15 and 7.5 %. Under the conditions of this test, m-XDI was found to be an extreme sensitiser. This study is considered to be the key study for classification (Huntingdon, 1997).

In a third test similar to OECD TG 406/EU B.6 (GPMT), m-XDI produced a sensitisation rate of 100 % with an intradermal induction dose of 0.01 % and re-challenge doses of 50 and 25 %. For a number of reasons (cf. left column in Table 10) this study is not considered sufficiently reliable to be used for classification and labelling. However, the reported results are consistent with m-XDI being an extreme sensitiser (Safepharm, 1998).

For a detailed summary of the above studies, the reader is referred to section 1.2 in Annex I to this dossier.

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

While no relevant human data on skin sensitisation caused by m-XDI were identified, a reliable GPMT demonstrated the potential of m-XDI to act as a skin sensitiser with extreme potency in guinea pigs. The other available animal tests were considered unreliable due to deficiencies in reporting and/or design (however, their results as reported were consistent with the proposed classification).

### 10.7.2 Comparison with the CLP criteria

According to the criteria given in Table 3.4.3 of the CLP regulation, skin sensitisers fall into Skin Sens. sub-category 1A based on the results from a GPMT test, if 30 % or more of the animals show a positive response at an intradermal induction concentration of ≤ 0.1 %. This criterion was fulfilled in all three available reliable GPMT tests in which at most time-points all treated animals showed a positive sensitisation reaction with intradermal induction concentrations of 0.1 or even 0.01 % (cf. Table 10).

Moreover, according to Table 3.7 of the CLP guidance (ECHA, 2017a) with 100 % sensitisation rate at intradermal induction concentrations ≤ 0.1 %, m-XDI qualifies as an "Extreme Sensitiser" for which the setting of a Specific Concentration Limit (SCL) of 0.001 % is recommended in Table 3.9 of the CLP guidance (ECHA, 2017a).

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

| Criteria acc. to Table 3.4.3 and Table 3.4.4 of the CLP regulation and Table 3.7 of the CLP guidance (ECHA, 2017a) | | Reference(s) | Sensitisation rate (%)/Intradermal induction dose (%) | Resulting Classification |
|---|---|---|---|---|
| **Skin Sens. 1A, Extreme** | ≥ 60 % responding at ≤ 0.1 % intradermal induction dose | (Huntingdon, 1997) | 100/0.01 | **Extreme sensitiser**<br>**Skin Sens. 1A**<br>**SCL 0.001 % (w/w)** |
| **Skin Sens. 1A, Strong** | ≥ 30 % responding at ≤ 0.1 % intradermal induction dose<br>or<br>≥ 60 % responding at > 0.1 – 1 % intradermal induction dose | | | |
| **Skin Sens. 1B, Moderate** | ≥ 30 - < 60 % responding at > 0.1 – 1 % intradermal induction dose<br>or<br>≥ 30 % responding at > 1 % intradermal induction dose | | | |

### 10.7.3 Conclusion on classification and labelling for skin sensitisation

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

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

No information on the skin sensitising potential of m-XDI in humans is available.

Four studies in guinea pigs are presented in the CLH report: one Guinea pig maximisation test (GPMT) study (Huntingdon, 1997), and three equivalent or similar to GPMT studies (Huntingdon, 1980; Safepharm, 1992, 1998). Out of these, only Huntingdon (1997) GPMT study was considered reliable (with restrictions, since no purity information was provided, and only summary was available), while other three studies were considered by the DS as unreliable (reliability 3) due to limitations in methodology and reporting.

The Huntingdon (1997) Guinea Pig Maximisation test (GPMT) was available to the DS only as a summary, provided by the REACH lead registrant for m-XDI. According to the summary, it is a GLP study, performed in 10 male Dunkin-Hartley Guinea pigs. The highest intradermal (0.01% in Alembicol D[5]) and topical induction concentrations (100%) applied in the range-finding study were chosen for the main experiment, since they caused only mild to moderate skin irritation and were well tolerated systemically. For topical challenge 15% and 7.5% (the highest non-irritant concentration and one lower concentration) were applied. Appropriate negative control was included (5 animals), and positive controls (hexyl cinnamic aldehyde, benzocaine, and 2-mercaptobenzothiazole) periodically checked the strain. Slight irritation was

[5] Fractionated coconut oil.

observed in test and control animals after intradermal inductions, and slight erythema after topical applications. No systemic effects were noted.

Positive reaction (necrosis, thickening, dryness and sloughing of the epidermis) occurred in all tested animals (10/10), at both challenge doses. Negative control animals did not show a positive reaction. Results were identical at 24 and 48 h post-challenge.

The DS concluded that a reliable GPMT demonstrated the potential of m-XDI to act as a skin sensitiser with extreme potency in guinea pigs (100% sensitisation rate at intradermal induction concentrations ≤ 0.1%, according to Table 3.7 of the CLP Guidance, 2017[6]), and proposed **Skin Sens. 1A**, with a Specific Concentration Limit (SCL) of 0.001% (as recommended for extreme potency skin sensitisers in Table 3.9 of the CLP guidance).

The other three available animal tests, for which also only summaries (as again provided by the REACH lead registrant for m-XDI) were available to the DS, were considered unreliable due to deficiencies in reporting and/or design. Nevertheless, their results were consistent with the proposed classification.

In the Huntingdon (1980) non-GLP, non-Guideline study, which was similar to a GPMT (OECD TG 406), dosing was performed by 0.01% m-XDI intradermal injections, topical induction with undiluted substance, and epi-cutaneous challenge with 20% m-XDI in acetone. Nine out of 10 exposed guinea pigs had a positive reaction (and none of 5 negative controls), which would trigger Skin Sens. 1A. However, elementary information on study methodology is missing, such as whether and which adjuvant was used, size of the treated area, and duration of topical exposure.

GLP has been claimed for the Safepharm (1992) GPMT study, although no purity and batch number of m-XDI were given. After intradermal induction with 0.1% of the test substance in Arachis oil BP (with Freund's Complete Adjuvant), topical induction with 75% test material, and epicutaneous challenge with 50% and 75% test material, all treated guinea pigs showed a positive sensitisation reaction 24 h and 48 h post-challenge, which would support Skin Sens. 1A classification. Nevertheless, skin erythema was also noted in negative controls, which could indicate irritation. As erythema scores were not reported, uncertainty remains about whether the test was performed in accordance with OECD TG 406, which requires non-irritant doses for the topical challenge.

The Safepharm (1998) study under GLP test was similar to OECD TG 406/EU B.6 (GPMT). In the study, m-XDI produced a sensitisation rate of 100% with an intradermal induction dose of 0.01% test substance in Arachis oil BP (with Freund's Complete Adjuvant), topical induction with undiluted test material, and re-challenge with 50% and 25% test material. The study, however, has serious deficiencies in design and reporting (i.e. results of the first challenge are not reported; re-challenge was performed with concentrations other than those used in the first challenge and much later than recommended in OECD TG 406, without explanation; 48 h after re-challenge, erythema could not be scored due to undisclosed "adverse reactions").

## Comments received during consultation

Three comments were received during the consultation from MSCAs, all supportive of the DS's proposal.

[6] ECHA Guidance on the Application of the CLP Criteria, Version 5.0, July 2017.

**Assessment and comparison with the classification criteria**

RAC agrees with the DS that the Huntingdon (1980), and Safepharm (1992 and 1998) studies are not reliable enough to be used for classification and labelling, but their results are in line with DS's proposed classification.

RAC considers that for regulatory purposes, summary of the key study Huntingdon (1997), a GLP study performed in accordance with OECD TG 406 (GPMT guideline), provides enough information on study methodology and results. The 2nd ATP[7] and ECHA CLP Guidance indicate that Skin Sens. sub-category 1A is applicable when there are ≥ 30% responding animals at ≤ 0.1% intradermal induction dose in a Guinea pig maximisation test. RAC, therefore, agrees with the DS that the results of this study justify **classification of m-XDI as Skin Sens. sub-category 1A (H317)**, since 100% tested animals had a positive reaction to m-XDI following 0.01% intradermal induction dose.

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

### 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

[7] Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures.

## 13 ADDITIONAL LABELLING

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

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

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

| **Additional labelling** |
| --- |
| According to the CLP regulation, Annex II, section 2.4, the following special rule for supplemental label elements shall apply for mixtures containing m-XDI:<br><br>*"Unless already identified on the label of the packaging, mixtures containing isocyanates (as monomers, oligomers, pre-polymers, 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-Reponse-Relationship

ESH: English smooth-hair

F: Female

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

$FEV_1$: Forced Expiratory Volume in one second

$FEV_1$%: $FEV_1$/FVC x 100

FVC: Forced vital capacity

GLP: Good laboratory practice

GP: Guinea pig

GPSA: Guinea pig serum albumin

HDI: Hexamethylene diisocyanate

HH: Human health

HMDI: "Hydrated MDI", 4'-methylenedicyclohexyl diisocyanate

HO: Head-only

IC: Isocyanurate

IDE: Intradermal

IF: Inflammation

IgE/IgG: Immunoglobulin E/G

INA: Intranasal

INH: Inhalation

IPDI: Isophoronediisocyanate

IPE: Intraperitoneal

IR & CSA: Information requirements and chemical safety assessment

ITR: Intratracheal

IUCL: Only IUCLID summary available

IVE: Intravenous

JEM: Job exposure matrix

LLNA: Local lymph node assay

LOD: Limit of detection

MDI: 4,4'-Methylenediphenyl-diisocyanate

M: Male

MIE: Molecular initiating event

MMF: Maximum mid-expiratory flow

MO: Mouse

NCO: Isocyanate functional group

NDI: 1,5-Naphthylene-diisocyanate

NO: Nose-only

n.s.: Not significant

OA: Occupational asthma

OR: Odds Ratio

OECD: Organization for Economic Co-Operation and Development

OVA: Ovalbumin

PEF(R): Peak expiratory flow (rate)

PHDI: Polymeric HDI

PIPDI: Polymeric IPDI

PMDI: Polymeric MDI

PR: Prevalence ratio

PU: Polyurethane

QSAR: Quantitative Structure-Activity Relationship(s)

RA: Rat

RB: Rabbit

REACH: Registration, evaluation, authorisation and restriction of chemicals

RF: Respiratory function

RR: Relative Risk

RS: Respiratory sensitisation

SCU: Subcutaneous

SS: Skin sensitisation

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

$TDI_{UC}$: TDI of unclear composition

TMI: Toluylenemono-isocyanate

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

TOE: Toepad inoculation

TOP: Topical

TWA: Time-weighted average

VP: Vapour

WB: Whole-body

m-XDI: 1,3-bis(isocyanatome-thyl)benzene