Committee for Risk Assessment

RAC

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

1,3-bis(1-isocyanato-1-methylethyl)benzene

EC Number: 220-474-4
CAS Number: 2778-42-9

CLH-O-0000006861-70-01/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

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CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),

Annex VI, Part 2

International Chemical Identification:

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

[m-TMXDI]

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

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

Index Number: **n.a.**

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

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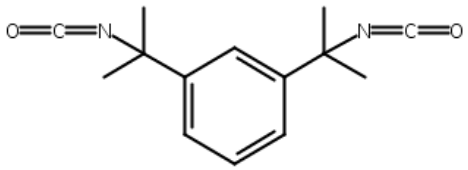
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,3-BIS(1-ISOCYANATO-1-METHYLETHYL)BENZENE

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(1-isocyanato-1-methylethyl)benzene
Other names (usual name, trade name, abbreviation)	meta-Tetramethylxylylenediisocyanate (m-TMXDI)
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	220-474-4
EC name (if available and appropriate)	1,3-bis(1-isocyanato-1-methylethyl)benzene
CAS number (if available)	2778-42-9
Other identity code (if available)	-
Molecular formula	$C_{14}H_{16}N_2O_2$
Structural formula	
SMILES notation (if available)	CC(C)(N=C=O)c1cccc(c1)C(C)(C)N=C=O
Molecular weight or molecular weight range	244.29 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
1,3-bis(1-isocyanato-1-methylethyl)benzene EC No. 220-474-4 CAS No. 2778-42-9	80-100	-	Skin Irrit. 2 (H315), Skin Sens. 1/1A (H317), Eye Irrit. 2 (H319), Acute Tox. 1 (H330), Resp. Sens. 1 (H334), STOT SE 3 (H335), STOT RE 1 (H372, Inhalation), Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Current, proposed, and resulting harmonised classification and labelling for m-TMXDI

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	1,3-bis(1-isocyanato-1-methylethyl)benzene; [m-TMXDI]	220-474-4	2778-42-9	Resp. Sens. 1 Skin Sens. 1A	H334 H317	GHS08 Dgr	H334 H317			
Resulting Annex VI entry if agreed by RAC and COM											

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

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Not applicable

RAC general comment
1,3-bis(1-isocyanato-1-methylethyl)benzene (m-TMXDI) is used in the production of polymers and has no current entry in Annex VI to the CLP Regulation. The CLH report has been prepared based on data submitted by the lead registrant in the REACH registration dossier for the 1,5-naphthylene diisocyanate (NDI), 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 Dossier Submitter (DS; Germany). In addition, SCOPUS and PubMed databases were searched for relevant literature, covering the period 2015 to 2017.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

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

5 IDENTIFIED USES

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

5.1 General

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

5.2 Consumer Uses

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

[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.

5.3 Article service life

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

5.4 Widespread use by professional workers

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

5.5 Formulation or re-packing

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

5.6 Uses at industrial sites

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

5.7 Manufacture

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

6 DATA SOURCES

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

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

7 PHYSICOCHEMICAL PROPERTIES

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

Property	Value	Comment (e.g. measured or estimated)
Physical state at 20 °C and 101.3 kPa	Liquid	-
Melting/freezing point	4 °C (melting range marked by onset and endset of melting peak: 4-12 °C)	Experimental result [OECD Guideline 102 (Melting point/Melting Range)]
Boiling point	DSC: 249.2 °C (1 atm); ebulliometer: 249.4 °C (1 atm)	Experimental result [OECD Guideline 103 (Boiling point/boiling range): DSC and ebulliometer]

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

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

To the best knowledge of the DS, no studies on the ADME properties of m-TMXDI are available. In the registration dossier, the lead registrant has provided some estimates based on the structure and physico-chemical properties, which, together with the DS comments and slight editorial amendments are presented in Table 6 below.

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

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

Property	Estimate by Registrant	DS Comment
	on exposure, a pool of isocyanate-conjugated albumin may persist in the circulatory system and reach a steady-state.	

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not assessed in this dossier

10.2 Acute toxicity - dermal route

Not assessed in this dossier

10.3 Acute toxicity - inhalation route

Not assessed in this dossier

10.4 Skin corrosion/irritation

Not assessed in this dossier

10.5 Serious eye damage/eye irritation

Not assessed in this dossier

10.6 Respiratory sensitisation

10.6.1 Endpoint definition and evaluation strategy

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

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

Article 9 of the CLP regulation specifies how the hazard information is evaluated to decide on classification. The strategy followed in this dossier is therefore characterised by presenting the few available substance-specific data for m-TMXDI which on their own do not suffice to classify it as a respiratory sensitiser. In a second step, these data are then complemented via a category approach by means of which the knowledge about the RS potential of the three most commonly used diisocyanates HDI, MDI, and TDI is read across to m-TMXDI. The use of category-based read-across for classification and labelling is covered by Article 5 1. (2) of the CLP regulation, which in turn refers to the methods listed in section 1 of REACH Annex XI. The category approach is justified in the following section. Finally, all available information is combined in an overall weight-of-evidence assessment in line with CLP Annex I, section 1.1.1.3.

10.6.2 Justification of the category approach

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

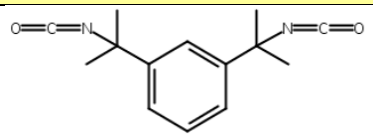
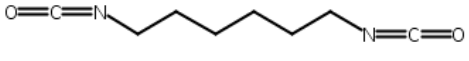
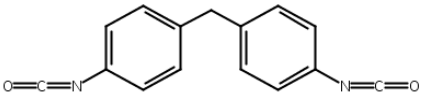
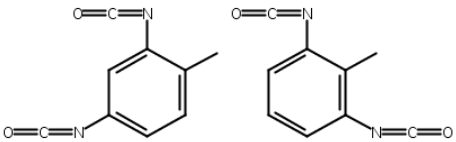
The approach relates to RAAF Scenario 6 (human health), i.e. the read-across hypothesis for the category is based on different compounds which have qualitatively similar properties, with no relevant variations in properties observed among source substances and the same strength predicted for the target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

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

10.6.2.2 AE C.1 Substance characterisation

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

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

EC Name; trivial name used in this report	EC No. CAS no.	CLH for sensitisation (Annex VI to CLP)	Structure
1,3-bis(1-isocyanato-1-methylethyl)benzene; m-TMXDI	220-474-4 2778-42-9	-	
Hexamethylene diisocyanate; HDI	212-485-8 822-06-0	Resp. Sens. 1 Skin Sens. 1	
4,4'-Methylenediphenyl diisocyanate; MDI[$]	202-966-0 101-68-8		
m-Tolylidene diisocyanate (80/20 mixture of 2,4-TDI and 2,6-TDI isomers); TDI[$]	247-722-4 26471-62-5		

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

10.6.2.3 AE C.2 Structural similarity and category hypothesis

As can be seen in Table 7, all members of the group (as well as the target substance) are monomeric diisocyanates, i.e. they share the structural feature of two isocyanate functional groups. The part of the molecular structure linking the two isocyanate groups may be variable.

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

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

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

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

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

10.6.2.6 AE C.5 Reliability and adequacy of the source data

This is addressed in the relevant parts of sections 10.6.4 and 10.6.5, as well as in Annex 1.

10.6.2.7 AE 6.1 Compounds the test organism is exposed to

In all studies used in this approach, the test organisms have been exposed to the source substances as described in Table 7 above.

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

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

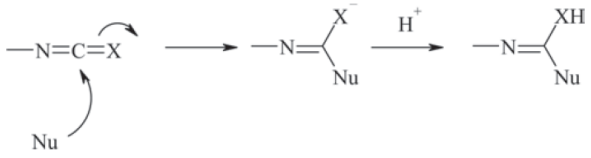


Figure 1: Acylation reaction for isocyanates (X = oxygen). Reproduced from (Enoch et al., 2011)

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

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

The potential reactivity of the diisocyanate source substances given in Table 7 above towards amino acids such as cysteine and lysine has been shown *in chemico* (Lalko et al., 2013).

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

Moreover, with respect to Table 7 above, the DS would like to point out that in terms of structure those molecular parts of the source substances separating the two isocyanate groups differ from each other, further highlighting that at least qualitatively the presence of the (two) isocyanate groups is the decisive factor for the RS potential, while the remaining molecular structure is of less importance (it might however have an impact

on the physico-chemical and ADME properties and therefore relative potency which is not addressed in this dossier).

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

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

10.6.2.10 AE C.6 Bias that influences the prediction

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

10.6.3 Data retrieval, evaluation, and presentation strategy

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

- Identify all studies in humans and animals for m-TMXDI, HDI, MDI, and TDI. Notably, numerous studies demonstrate the ability of diisocyanates to cause symptoms of RS also after dermal exposure (cf. the restriction report for diisocyanates recently submitted by the German MSCA[3]), however, since the definition from the CLP regulation cited in section 10.6.1 clearly asks for inhalation exposure, only studies along this route were evaluated for the current dossier.
- Evaluate and present the relevant human data, first for m-TMXDI, then for the three source substances HDI, MDI, and TDI.
- Filter animal data for relevance according to predefined criteria (cf. section 10.6.5).
- Evaluate and present the relevant animal data, first for m-TMXDI, then for the three source substances HDI, MDI, and TDI.
- Summarise, compare to the CLP criteria and conclude on a possible potential for RS.

10.6.4 Human data

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

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

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

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

more general term "airway hyperresponsiveness/hyperreagibility (AHR)" is used interchangeably) to non-specific stressors including e.g. other chemicals such as methacholine, cold, dust, or physical exercise that can last for years even in the absence of continued exposure, and complete recovery of lung function may never be achieved (Johnson et al., 2004a).

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

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

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

10.6.4.1 Human data for the target substance m-TMXDI

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

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

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

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

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

More than 100 case reports and epidemiological studies have been evaluated. An overview of this evaluation is provided in Annex I, Table 2 (case reports) and Tables 3-8 (epidemiological studies). The case reports

provide overwhelming proof that humans exposed to the source substances HDI, MDI, and/or TDI may suffer from a broad spectrum of respiratory effects including asthma and pathological changes of the airways. Also a number of fatal cases have been reported, albeit not in recent years. While during the early stages of the development of the disease, respiratory symptoms may eventually be reversed upon removal from exposure, an irreversible remodelling of the airways will eventually take place when exposure is continued. On the other hand these case reports do not allow for an assessment of the frequency of occurrence of respiratory sensitisation to m-TMXDI in the human population as they feature only a small number of patients and it is not known which fraction of all exposed persons is affected (and which fraction of the affected is reported). They are therefore not suited for sub-categorisation. In addition, no harmonised approach for sub-categorising respiratory sensitisers is available yet.

An overview of epidemiological studies on diisocyanates and respiratory effects conducted until today with short study descriptions and results is given in Annex 1, Tables 3-8. Despite a large number of available studies, none of these studies is eligible for deriving a reliable Exposure-Response-Relationship (ERR) due to limitations of the studies. This is also inherent in the mechanism of the disease. No study overcomes the problem that sensitive predictive markers for diisocyanate sensitisation are missing and that dermal exposure as well as inhalation peak exposure likely contribute to the induction of sensitisation, but cannot be assessed appropriately to date.

10.6.5 Animal data

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

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

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

As a consequence, the animal database available for the three source substances and the target substance m-TMXDI has been evaluated and filtered for relevant studies (the complete list of studies is available in Table 9 in Annex I to this dossier). To that end, studies were discarded which used induction routes other than the inhalation route (or mixed designs including e.g. intradermal and inhalation induction). Only true inhalation studies were accepted, while those using intranasal exposure, intratracheal instillation, or oropharyngeal administration were not considered any further.

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

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

Animal study designs for respiratory sensitisation have been manifold, involving a variety of species, protocols, and target endpoints, and a standardised protocol with regulatory acceptance is still missing. Therefore a negative result from an animal experiment on RS is not suitable to exclude the need for classification and labelling. Consequently, for the read-across assessment the evaluation concentrated on data providing a positive indication of respiratory sensitisation, therefore for HDI, MDI, and TDI only studies reporting the presence of one or more relevant effects were selected for further processing. Where several experiments were reported in one study report, only those with effects were processed further. For studies with m-TMXDI, however, all studies meeting the above criteria (inhalation route, reliability) are described below, regardless of whether an effect was observed or not.

Finally, studies using agents other than m-TMXDI or the three source substances (as per Table 7) in their monomeric form, i.e. their prepolymers, breakdown products or protein conjugates or other isomers for induction, or for which the exact identity was unclear, were also dismissed.

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

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

In the end, a total of 39 experiments from 21 study reports, performed in guinea pigs, mice, and rats qualified for further evaluation. Table 8 provides an overview of the number of studies and their distribution over the different substances and rodent species.

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

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

10.6.5.1 Animal data for the target substance m-TMXDI

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

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

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

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

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

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

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

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

Table 10 provides an overview of the results of the experiments with HDI, MDI, and TDI selected for further evaluation regarding the potential of these substances to cause respiratory sensitisation.

Table 10: Studies for evaluating the potential of the source substances HDI, MDI, and TDI to cause RS in rodents following exposure via the inhalation route (sorted by species and year, see section 15 for abbreviations)

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

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

10.6.5.2.1 Guinea pigs

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

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

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

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

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

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

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

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

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

10.6.5.2.2 Mice

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

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

Arts and colleagues used a "respiratory local lymph node assay", i.e. a study protocol in which male Balb/c mice were first exposed once per day on three consecutive days to HDI or TDI by inhalation,

[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.

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

Although providing some evidence of specific antibody formation, human data for m-TMXDI are by themselves not sufficient for classifying this substance as a respiratory sensitiser. However, a large database of human data on the source substances HDI, MDI, and TDI provides undeniable proof that these substances are able to cause RS in humans and are therefore rightfully listed as Resp. Sens. 1 in Annex VI to the CLP regulation.

10.6.6.2 Animal data

Again the available data for m-TMXDI give some indication of substance-related antibody formation, but are otherwise not sufficient on its own to justify classification for RS. In contrast, exposure to the three source substances by inhalation was shown to trigger RS in a variety of rodent species as demonstrated by the production of specific antibodies, impairment of respiratory function, and characteristic inflammation markers in BALF. Observed respiratory symptoms (increased respiratory rate, effects on respiratory flow, laboured breathing etc.) resemble those seen in humans with asthma.

Skin sensitisation has also been observed following induction via inhalation.

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

10.6.7 Comparison with the CLP criteria

10.6.7.1 Human data

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

Since for m-TMXDI, only one study in humans is available which, however, is not adequate for classification and labelling, a category approach is used for classification in accordance with CLP Article 5 1. (2) referring to REACH Annex XI, section 1. Numerous case reports and epidemiological studies with the category source substances HDI, MDI, and TDI evaluated for this dossier report positive bronchial provocation tests with these substances. In addition, many of the other criteria mentioned above are met by these reports.

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

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

10.6.7.2 Animal data

One study with m-TMXDI, which, however is considered to be of limited reliability, documented the production of specific antibodies following the exposure of guinea pigs to m-TMXDI by inhalation. In addition, several studies in guinea pigs, mice, and rats with the source substances HDI, MDI, and TDI were identified in which the production of specific antibodies and the impairment of pulmonary function as a consequence of exposure to diisocyanates via inhalation were demonstrated.

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

10.6.8 Conclusion on classification and labelling for respiratory sensitisation

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

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

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

RAC evaluation of respiratory sensitisation
Summary of the Dossier Submitter's proposal The DS proposed to classify 1,3-bis(1-isocyanato-1-methylethyl)benzene (m-TMXDI) as Resp. Sens. 1; H334. Currently, m-TMXDI has no harmonised classification in Annex VI to the CLP Regulation.

There are no specific reliable respiratory sensitisation data available for m-TMXDI that would be sufficient on their own to evaluate the need for classification. Therefore, the proposed harmonised classification was based on a weight of evidence assessment of the available data and read across.

Only the three most commonly used source substances were used for read across as most of the published literature on diisocyanates is related to these: hexamethylene diisocyanate (HDI; CAS number 822-06-0), 4,4'-methylenediphenyl diisocyanate (MDI, CAS number 101-68-8) and m-tolylidene diisocyanate (TDI; CAS number 26471-62-5; 80/20 mixture of 2,4-TDI and 2,6-TDI isomers). They all have harmonised classifications as Resp. Sens. 1; H334. In addition, the DS noted that also several other diisocyanates have been self-classified as respiratory sensitisers. 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 read across target substance m-TMXDI

The DS identified only one report addressing potential respiratory sensitisation in humans by m-TMXDI. Grammer *et al.* (1993) reported an evaluation of 96 workers from facilities manufacturing or using m-TMXDI. While ca. 40% of the workers reported to have experienced irritation of the upper respiratory tract and/or the eyes, no workers with new asthma or other severe respiratory symptoms were identified. Two workers reported exacerbation of a previously existing asthmatic disease. Serological assessments showed m-TMXDI-specific IgE antibodies in one worker and m-TMXDI-specific IgG antibodies in eight workers. Overall, 12% of the workers exposed to estimated maximum concentrations of 0.4 to 10.2 ppb tested positive for m-TMXDI-specific antibodies.

However, the DS identified several significant limitations in the report, including the following:

- the symptoms were only self-reported and respiratory function tests were not performed;
- there was no follow-up of the workers who tested positive for specific antibodies;
- no information was provided on the possible origin of asthma in the two reported exacerbation cases;
- low estimated exposure levels and unknown true exposure level;
- no information on whether all the surveyed workers had worked in the factory over the whole study period (1984-1988);
- no information on whether during this period workers had left the factory, in particular after the early phase of factory setup, which was identified by the authors as a phase of potentially higher exposure, and if so, whether these workers had shown symptoms of respiratory disease.

The DS concluded that as evidence of immunological reactions in several workers was shown, the results do not demonstrate the absence of a potential of m-TMXDI to cause respiratory sensitisation in humans. They also concluded that the results are not suitable

to rank m-TMXDI as a "low" or "lower than other diisocyanates" potency respiratory sensitiser, as the authors of the study had concluded.

Animal data on the read across target substance m-TMXDI

There are no internationally recognised *in vivo* test methods for identification of respiratory sensitisation. Animal studies were considered by the DS to be relevant for the classification only if the induction route was truly inhalation. Studies using other routes of induction or mixed routes were discarded. Furthermore, studies were considered unreliable and excluded from the assessment if 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.

Regarding m-TMXDI, all studies meeting the above criteria (inhalation route, reliability) were included, regardless of whether an effect was observed or not. Three inhalation studies performed in guinea pigs were identified and assessed by the DS, summarised in the table below. For all of these studies, only IUCLID summaries submitted by the REACH lead registrant were available. Two of these studies were considered not reliable (quality issues in design and reporting, assessed only a limited spectrum of effect parameters). The third study (Union Carbide, 1988) was considered reliable with restrictions.

The DS concluded that overall, beyond a weak indication of possible antibody formation of unknown type, none of these studies can reliably contribute to the identification of m-TMXDI as a respiratory sensitiser. They also noted that they be used to prove the absence of respiratory sensitisation potential in humans. As mentioned before, due to lack of a standardised animal test design with regulatory acceptance, negative findings from such experiments cannot be used to exclude the need for classification and labelling for RS.

Table. *Summary by the DS of the animal studies on sensitisation after induction via inhalation with m-TMXDI (from Table 9 in the CLH report).*

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

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

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

More than 100 case reports and epidemiological studies were evaluated by the DS. The literature outlined in tables 2-8 of Annex I of the CLH report consistently demonstrate the potential of HDI, MDI and TDI to cause respiratory sensitisation in humans.

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. In addition, 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 in the human population; they feature only a small number of patients and it is not known, which fraction of all exposed individuals is affected (and which fraction of the affected is reported). The case reports are therefore not suited for sub-categorisation. In addition, no harmonised approach for sub-categorising respiratory sensitisers is available yet.

According to the DS, despite the large number of available epidemiological studies, none of them is eligible for deriving a reliable Exposure-Response-Relationship 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.

Patients with diisocyanate-induced asthma display both early (seconds to minutes) and delayed (up to several hours) hypersensitivity. However, the prevalence of delayed responses is as high as 70% (Niimi *et al.*, 1996). A particular concern is the delay between onset of (low-level) exposure at work and the manifestation of the asthmatic symptoms, which may be as long as several years after the start of exposure. In addition, patients often develop persistent bronchial 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, and complete recovery of lung function may never be achieved (Johnson *et al.*, 2004a).

Animal data for the source substances HDI, MDI and TDI

The same criteria as described above (under *Animal data for the target substance m-TMXDI*) were used by the DS to select the studies that were considered relevant and reliable for the classification. In addition, regarding the source substances, the DS noted that 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, the DS noted that a negative result from an animal experiment on respiratory sensitisation 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 by the DS for further processing. Where several experiments were reported in one study report, only those with effects were processed further.

For HDI, MDI and TDI, 36 experiments from 18 study reports qualified for further evaluation, 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 broncho-alveolar lavage fluid (BALF). Observed respiratory symptoms (increased respiratory rate, effects on respiratory flow, laboured breathing etc.) resemble those seen in humans with asthma. In addition, skin sensitisation has also been observed following induction via inhalation. However, the interdependencies and quantitative contributions to sensitisation of factors such as the species and strain used, concentration and total dose received upon induction, or the temporal pattern of dosing are still poorly understood.

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

The read across 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). In this scenario, the read across hypothesis was based on different compounds that have qualitatively similar properties, with no relevant variations in properties observed among source substances and the same strength predicted for the target substance. All assessment elements relevant to the RAAF Scenario 6 (human health) were considered by the DS.

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

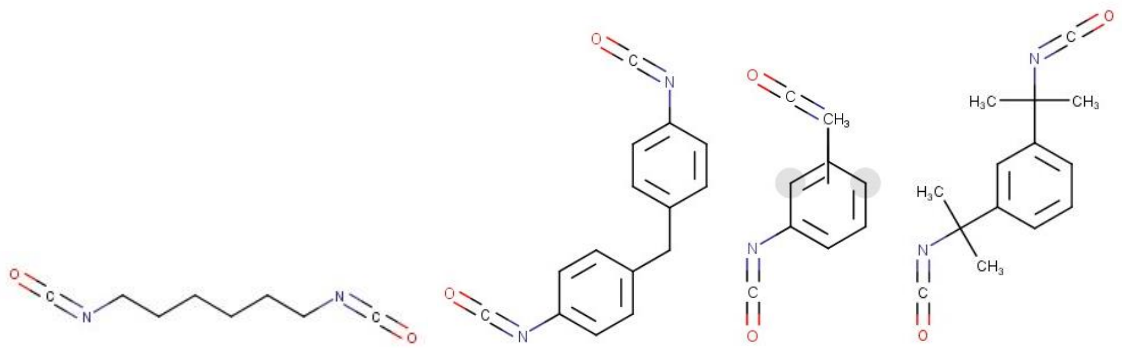


Figure. *The structures of HDI, MDI, TDI and m-TMXDI, respectively, from left to right.*

The isocyanate 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, that similarly to skin sensitisation, covalent binding of electrophiles to proteins in the lung marks a molecular initiating event and that for isocyanates, an acylation type reaction between electrophilic N=C=O functional groups and nucleophilic protein moieties may occur, leading to protein adducts (Enoch *et al.*, 2009; 2011; 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 highly in this respect (Agius *et al.*, 2000). The potential reactivity of HDI, MDI and TDI towards amino acids has been shown *in chemico* (Lalko *et al.*, 2013).

Moreover, the DS noted that at least the qualitative respiratory sensitising potential of HDI, MDI and TDI appears to be dependent on the diisocyanate structure. The variations in the molecular structure connecting the two 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 is no validated test method for respiratory sensitisation, and therefore compounds are typically classified for Resp. Sens. based on human data, with supportive evidence from e.g. animal data.

For m-TMXDI, specific antibody formation in humans (workers) and an indication of possible antibody formation of unknown type in guinea pigs has been shown. While these data provide support for the proposed classification, they are not sufficient on their own to warrant classification for respiratory sensitisation. Furthermore, data on skin sensitisation (discussed below) demonstrates that m-TMXDI has sensitising properties

For the source substances HDI, MDI and TDI, numerous case reports and epidemiological studies consistently demonstrate potential to cause respiratory sensitisation in humans. *In vivo* studies provide additional support. Consequently, all three source substances have existing harmonised classification as Resp. Sens. 1; H334, as do 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 considered an alert for respiratory sensitisation.

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

CLP, Annex I, section 3.4.2.1.2.3 states that the evidence required to demonstrate respiratory sensitisation in humans *"could be: (a) clinical history and data from appropriate lung function tests related to exposure to the substance, confirmed by other supportive evidence which may include: (i) in vivo immunological test (e.g. skin prick test); (ii) in vitro immunological test (e.g. serological analysis); (iii) studies that indicate other specific hypersensitivity reactions where immunological mechanisms of action have not been proven, e.g. repeated low-level irritation, pharmacologically mediated effects;* ***(iv) a chemical structure related to substances known to cause respiratory hypersensitivity****; (b) data from one or more positive bronchial challenge tests with the substance conducted according to accepted guidelines for the determination of a specific hypersensitivity reaction".* Furthermore, section 3.4.2.1.2.5 notes that *"the results of positive bronchial challenge tests are considered to provide sufficient evidence for classification on their own"* (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 adequate. In addition, RAC agrees with the justification for a category approach using read across (based on human and non-human data) from the known respiratory sensitisers HDI, MDI and TDI to the target substance m-TMXDI. RAC also agrees that it is not possible to sub sub-categorise m-TMXDI into 1A or 1B, as no reliable data on the potency of either m-TMXDI 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-TMXDI.

10.7 Skin sensitisation

To the knowledge of the DS, no studies of the skin sensitising potential of m-TMXDI in humans are available. However, skin sensitisation test data in animals (BRC, 1981), summarised in the tables below as well as in Annex I to this dossier, are available for m-TMXDI, which are sufficient for classification and labelling. Therefore, in this case read-across from other diisocyanates is not necessary. Nevertheless it is stressed that all diisocyanates currently classified as respiratory sensitisers in Annex VI to the CLP

regulation also are classified as skin sensitisers or, in the case of naphthylene diisocyanate (NDI, CAS 3173-72-6) have data showing their skin sensitisation potential.

Table 11: Summary table of animal studies on skin sensitisation

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

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

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

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

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

In a skin sensitisation test with m-TMXDI similar to the Buehler protocol (BRC, 1981), between 50 and 100 % of the treated animals showed a positive response both 24 and 48 h post-challenge, depending on the challenge concentration (cf. Table 12 above). For all of the four highest challenge doses (0.0125-0.1 %) responses were 70 % or greater (but cf. footnote to Table 12). Upon re-challenge 24 or 48 h post-challenge, no positive reactions were reported. The reason for this is unclear, but it is noted that also the positive control (IPDI) gave only lower or no positive results upon re-challenge which might indicate experimental problems at the re-challenge step. In addition, the test protocol used showed some deviations from the Buehler test method as laid out in OECD TG 406. In the view of the DS, those deviations (less animals used, only one instead of three induction exposures, non-occlusive exposure,

early challenge) all tend to decrease the sensitivity of the test and a negative test result would not have been acceptable in this case. However, since clear positive results were obtained, the DS rates this study as "reliable with restrictions" or Klimisch code 2.

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

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

10.7.1 Comparison with the CLP criteria

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

10.7.2 Conclusion on classification and labelling for skin sensitisation

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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No information on the skin sensitising potential of m-TMXDI in humans is available.
One animal study is available (Biosphere Research Centre. Cytec Industries, unpublished, BRC, 1981), similar to the Buehler test (OECD TG 406), for which GLP compliance has been claimed.

The study was performed in Hartley guinea pigs of unspecified gender, 10 per group. Induction was performed by epicutaneous (non-occlusive) application of m-TMXDI (purity 91.58%) at 0.36 molar concentration (around 9% w/v) in olive oil, and challenge and re-challenge with 0, 0.10, 0.05, 0.025, 0.0125 and 0.00625 molar dilutions (units expressed as percentage in the CLH Report), 5 and 14 days after single induction application, epicutaneously (open application). Isophoronediisocyanate (IPDI) was used as a positive control. Prior to the induction application, the primary irritation potential was determined.

The DS recognised significant deviations from OECD TG 406 protocol, and other limitations in the study methodology and reporting, as follows:

- only a summary of the study is available;
- only 10 animals per group were used;
- exposure was non-occlusive;
- there was only one induction application;
- challenge was performed earlier than days 27-29;
- irritant doses were also used for challenge (the concentration used for the challenge exposure should be the highest non-irritating dose);
- individual scores for skin changes after challenge or re-challenge are not given in the summary;
- upon re-challenge (24h or 48h post-challenge), no positive reactions were reported;
- positive control (IPDI) gave only lower or no positive results upon re-challenge.

The DS pointed out that although the reason for negative results in re-challenge is unclear, the positive control gave only lower or no positive results upon re-challenge which might indicate experimental problems at the re-challenge step. Furthermore, the deviations from OECD TG 406, including only one instead of three induction exposures, non-occlusive exposure and early challenge, could decrease the sensitivity of the test, and a negative test result would not have been acceptable in this case.
Due to clear positive results obtained (table below), the DS rated the study as "reliable with restrictions" or Klimisch score 2, and proposed **Skin Sens. 1A** (H317: May cause an allergic skin reaction). Namely, according to the criteria given in Table 3.4.3 of the CLP regulation, skin sensitisers fall into category 1A based on the results from a Buehler test, if 60% or more of the animals show a positive response at a topical induction concentration of > 0.2 to ≤ 20%. This criterion was fulfilled for four of the five challenge doses tested (0.0125% - 0.1%) at the first reading, and for all tested doses at the second reading.

***Table**. Results from a study on skin sensitisation with m-TMXDI (BRC, 1981) (Table 12 from CLH Report)*

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

* According to the summary in the REACH registration dossier, these doses were slightly irritant (grade 1 erythema) in 2/5 females and irritant (grade 2 erythema) in 1/5 males tested during the primary skin irritation phase.

***Table**. Mean skin irritation scores (BRC, 1981) (Table 12 in Annex 1; the values have been reproduced by the DS from the summary presented in the REACH registration dossier)*

Primary Skin Irritation Phase:											
Concentration	0.1		0.05		0.025		0.0125		0.00625		
	Er	Ed	Er	Ed	Er	Ed	Er	Ed	Er	Ed	Er
IPDI (24 h)	0.6	0.0	0.4	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2
IPDI (48 h)	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.0
11583B15 (24 h)	0.8	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
11583B15 (48 h)	0.4	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Challenge Phase:											
IPDI (24 h)	2.7	0.5	2.1	0.0	1.5	0.0	1.1	0.0	0.9	0.0	0.0
IPDI (48 h)	1.9	0.0	1.9	0.0	1.7	0.0	1.2	0.0	0.9	0.0	0.0
11583B15 (24 h)	2.3	0.2	2.1	0.2	0.7	0.0	1.1	0.0	0.5	0.0	0.0
11583B15 (48 h)	2.1	0.0	2.0	0.0	1.0	0.0	1.2	0.0	0.8	0.0	0.2
Rechallenge Phase:											
A-IPDI (24 h)	0.9	0.0	0.8	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
A-IPDI (48 h)	0.7	0.0	0.6	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
B-IPDI (24 h)	0.5	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
B-IPDI (48 h)	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11583B15 (24 h)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11583B15 (48 h)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

11583B15 = m-TMXDI; A: Animals treated with IPDI during induction; B: Animals treated with m-TMXDI during induction; * Vehicle (olive oil) only; Er: Erythema; Ed: Oedema

A specific concentration limit (SCL) was not proposed by the Dossier Submitter.

Comments received during consultation

Three comments were received during the consultation (from MSCAs). Although they pointed out limitations of the study and some further unclarities in the study reporting, all were supportive of the DS's proposal.

Assessment and comparison with the classification criteria

The results of BRC (1981) study, presented in two tables above, indicate strong sensitising potential for m-TMXDI (positive reaction in up to 100% of the animals tested, both at the 24h and 48h reading). Mean scores for the challenge phase stated in the table above showed that the reaction did not diminish at the second reading, indicating a sensitisation rather than irritation reaction. Based on these results, classification as Skin Sens. Cat. 1A would be warranted, according to the criteria given in Table 3.4.3 of the CLP Regulation. However, the study had numerous limitations, which are listed above. Additionally, while the induction dose was expressed only as a molar concentration, as commented during the Consultation, it is not clear in which units the challenge and re-challenge doses were expressed – percentage (e.g. % w/v), percentage molar concentration or molar dilution, since all these units are used interchangeably in

the CLH Report, Annex 1 and the REACH registration dossier. Further clarification on this issue is not possible, since only a summary from the REACH registration dossier is available.

RAC, therefore, considers that an assessment of the skin sensitisation potential of m-TMXDI cannot be based solely on this study, and has conducted a weight-of-evidence approach in which read across from other diisocyanates have also been used.

RAC has conducted the same read across procedure as done for respiratory sensitisation endpoint for this substance, i.e. based on the category approach and structural similarity to monomeric diisocyanates, according to the RAAF Scenario 6 (human health). The read across hypothesis is based on different compounds that have qualitatively similar properties, with no relevant variations in properties observed among source substances and the same strength predicted for the target substance.

The justification for the read across for respiratory sensitisation endpoint provided in the sections above (*RAC evaluation of respiratory sensitisation*) applies in much the same way to skin sensitisation. Namely, the available evidence demonstrates that the presence of two isocyanate groups already sufficiently indicates sensitisation potential, whereas the nature of the chemical structure connecting the two isocyanate groups is of less importance. The three most commonly used diisocyanate substances, which all have harmonised classifications as Resp. Sens. 1; H334, and Skin. Sens. 1; H317, were used as source substances, because most of the published literature on diisocyanates is related to these (HDI, MDI and TDI). Moreover, as shown in Table 9 of the CLH Report for 2,4-diisocyanato-1,3,5-triisopropylbenzene (TRIDI), there are more diisocyanates that are classified both as Resp. Sens. 1 and Skin Sens. 1 (including o-(p-isocyanatobenzyl)phenyl isocyanate, 4,4'-methylenedi(cyclohexyl isocyanate), 3-isocyanatomethyl-3,5,5-trimethylcyclohexyl isocyanate, 4-methyl-m-phenylene diisocyanate, 2-methyl-m-phenylene diisocyanate, 4,4'-methylene bis(3-chloro-2,6-diethylphenylisocyanate), 2,5-bis-isocyanatomethylbicyclo[2.2.1]heptane, S-(3-trimethoxysilyl)propyl 19-isocyanato-11-(6-isocyanatohexyl)-10,12-dioxo-2,9,11,13-tetraazanonadecanethioate).

In addition, based on substance-specific animal data, RAC proposes to classify m-XDI (EC 222-852-4) and NDI (EC 221-641-4) as strong or even extreme skin sensitisers.

In conclusion, based on weight-of-evidence approach, which took into account:

- that the data for m-TMXDI as such, although uncertain, support 1A (i.e. strong positive response in a Buehler-like study (BRC, 1981) with significant limitations);
- read across from the known Cat. 1 skin sensitisers HDI, MDI and TDI, to the target substance m-TMXDI;
- strong or even extreme skin sensitising property of m-XDI and NDI, for which Skin Sens. Cat. 1A has been proposed by RAC, based on substance-specific experimental data;
- the close structural similarity between m-TMXDI and the strong sensitiser m-XDI;
- the likelihood that all isocyanates are strong sensitisers;[5]

[5] RAC notes that subcategorisation (1A) is not proposed for another diisocyanate evaluated by RAC, 2,4,6-triisopropyl-m-phenylene diisocyanate (TRIDI), due to complete lack of experimental data for this substance. In the case of m-TMXDI,

RAC considers that classification as **Skin Sens. Cat. 1A**; H317 is warranted for m-TMXDI.

An SCL is not proposed, since RAC considers that numerous limitations in the experimental data for m-TMXDI (BRC, 1981) render it insufficiently reliable to support setting an SCL.

10.8 Germ cell mutagenicity

Not relevant for this dossier

10.9 Carcinogenicity

Not relevant for this dossier

10.10 Reproductive toxicity

Not relevant for this dossier

10.11 Specific target organ toxicity-single exposure

Not relevant for this dossier

10.12 Specific target organ toxicity-repeated exposure

Not relevant for this dossier

10.13 Aspiration hazard

Not relevant for this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not relevant for this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not relevant for this dossier

13 ADDITIONAL LABELLING

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

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

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

however, experimental data exist, and although there are numerous limitations, the data indicate strong sensiting potential of m-TMXDI, as stated above.

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****."*

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ECHA 2018. Committee for Risk Assessment (RAC) Committee for Socio-economic Analysis (SEAC) Annex to the Background document to the Opinion on the Annex XV dossier proposing restrictions on diisocyanates.

15 LIST OF ABBREVIATIONS

AB: Antibodies

ADME: Absorption, distribution, metabolism, and excretion

AE: Aerosol

AHR: Airway hyperresponsiveness

AOP: Adverse outcome pathway

BAL(F): Bronchoalveolar lavage (fluid)

BHR: Bronchial hyperresponsiveness

BT: Biuret

CLH: Harmonised classification and labelling

CLP: Classification, labelling, and packaging

DO: Dog

DS: Dossier submitter

DSC: Differential scanning calorimetry

DH: Dunkin-Hartley

ECHA: European Chemicals Agency

ERR: Exposure-Response-Relationship

ESH: English smooth-hair

F: Female

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

FEV_1: Forced Expiratory Volume in one second

FEV_1 %: FEV_1/FVC x 100

FVC: Forced vital capacity

GLP: Good laboratory practice

GP: Guinea pig

GPSA: Guinea pig serum albumin

HDI: Hexamethylene diisocyanate

HH: Human health

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

HO: Head-only

IC: Isocyanurate

IDE: Intradermal

IF: Inflammation

IgE/IgG: Immunoglobulin E/G

INA: Intranasal

INH: Inhalation

IPDI: Isophorone-diisocyanate

IPE: Intraperitoneal

IR & CSA: Information requirements and chemical safety assessment

ITR: Intratracheal

IUCL: Only IUCLID summary available

IVE: Intravenous

JEM: Job exposure matrix

LLNA: Local lymph node assay

LOD: Limit of detection

MDI: 4,4'-Methylene-diphenyldiisocyanate

M: Male

MIE: Molecular initiating event

MMF: Maximum mid-expiratory flow

MO: Mouse

NCO: Isocyanate functional group

NDI: 1,5-Naphthylene-diisocyanate

NO: Nose-only

n.s.: Not significant

OA: Occupational asthma

OR: Odds Ratio

OECD: Organization for Economic Co-Operation and Development

OVA: Ovalbumin

PEF(R): Peak expiratory flow (rate)

PHDI: Polymeric HDI

PIPDI: Polymeric IPDI

PMDI: Polymeric MDI

PR: Prevalence ratio

PU: Polyurethane

QSAR: Quantitative Structure-Activity Relationship(s)

RA: Rat

RB: Rabbit

REACH: Registration, evaluation, authorisation and restriction of chemicals

RF: Respiratory function

RR: Relative Risk

RS: Respiratory sensitisation

SCU: Subcutaneous

SS: Skin sensitisation

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

TDI_{UC}: TDI of unclear composition

TMI: Toluylenemono-isocyanate

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

TOE: Toepad inoculation

TOP: Topical

TWA: Time-weighted average

VP: Vapour

WB: Whole-body

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

[m-TMXDI]

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

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

Index Number: **n.a.**

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Version number: **2.0**

Date: **July 2019**

CONTENTS

Note to the reader: For an explanation of the abbreviations used in this Annex, please refer to the list of abbreviations provided in the main dossier.

1 HEALTH HAZARDS

1.1 Respiratory sensitisation

1.1.1 Human data for m-TMXDI

1.1.1.1 Case study (Grammer et al., 1993)

Study reference

Grammer L.C., Shaughnessy M.A., and Davis R.A. (1993): Exposure to TMXDI (meta) aliphatic isocyanate and TMI (meta) unsaturated aliphatic isocyanate. Clinical and immunological evaluation of 96 workers. J Occup Med 35 (3), 287-290

The text below is reproduced from the original publication, with slight editorial modifications by the DS. For a critical evaluation of the results by the DS, the reader is referred to the main part of this dossier.

Study design

Test type

Case-control study

Substances investigated

m-TMXDI, m-TMI (m-toluylmonoisocyanate).

Study population

The study population consisted of 96 workers at facilities that produced or used m-TMXDI or m-TMI. Two facilities were laboratories where the preliminary manufacturing experiments were performed, while a third facility was the start-up plant where m-TMXDI was manufactured by a continuous process and piped into 55-gallon drums.

Exposure

Personal sampling for m-TMI and m-TMXDI was performed during routine running conditions several times in 1984, 1985, and 1988. For m-TMXDI, a minimum sample size of 240 L was obtained using a Gelman 37-mm type AE glass-fiber filter treated with a nitro-reagent in a closed-face cassette. A persona| sampling pump operating at 1-2 L/min for 4-8 h was used in the sampling train that drew air from the proximity of the worker's breathing zone. The m-TMXDI vapour was collected and converted to a stable urethane derivative on glass-fiber filters treated with 4- Nitro-N-propylbenzylamine (nitro reagent). The derivatised material was desorbed from the filter, followed by high-performance liquid chromatography analysis. For m-TMI, sampling procedures were similar to those outlined for m-TMXDI except that the 37-mm glass-fiber filter was tested with 1-(2-pyridyl)piperazine. Storage conditions and stability were still under investigation and thus levels of TMI were not available at the time of the publication.

Questionnaires

The questionnaire used was developed by the American Thoracic Society questionnaire and was modified to assess the relationship of symptoms to workplace exposures. Prior to any knowledge of the antibody data, two physician graders at Northwestern University determined the diagnoses; there was agreement between the physician evaluators in all diagnoses. In late 1988 or early 1989, questionnaires were completed by the plant physician or nurse for each worker.

Conjugation of TMXDI-HSA and TMI-HSA

m-TMXDI or m-TMI were covalently linked to HSA using a modification of a previously described method. To document that conjugation had occurred, immunoelectrophoresis was performed (IEP, Calbiochem-Behring, La Jolla, CA). In addition, to determine the epitope density of the conjugates, an assay for free amino groups was performed.

Enzyme-Linked Immunosorbent Assay (ELISA)

In late 1988 or early 1989, a serum sample was obtained from each of 96 workers in three facilities, all of which produced or used m-TMXDI and m-TMI. Sera were stored frozen at -20 °C until the immunoassays were performed. The ELISA procedure was performed according to previously described protocols. Polystyrene Immulon micro-ELISA plate wells (Greiner and Sons, Nürtingen) were coated with m-TMXDI-HSA or m-TMI-HSA at a concentration of 100 g/mL in carbonate buffer (pH 9.6). A volume of 200 µL was used for all steps in the assays; washes between steps in the assays were performed three times with phosphate buffered saline (PBS) containing 0.05 % Tween 20 (Sigma Chemical Co, St. Louis, Mo). Appro- priate dilutions of sera in PBS-Tween were incubated in the plates for 60 min at 37 °C. Rabbit antihuman IgG or IgE (Calbiochem Behring) diluted 1:1000 in PBS-Tween was incubated for 45 minutes at 37 °C. Goat antirabbit IgG conjugated to alkaline phosphatase (Sigma) diluted 1:1000 in PBS-Tween was incubated at 37 °C for 60 min. P-nitrophenyl phosphate (Sigma 104 phosphatase) l mg/mL in diethanolamine buffer (pH 9.8) was added to each well and then the optical density was determined at 405 nm on a BioTek Model EL312 automated ELISA reader (Bio-Tek Instruments, Winooski, Vt). The reaction was allowed to proceed until the positive control serum attained a predetermined value. Any serum resulting in an optical density greater than twice that of the negative control sera was assayed at additional serum dilutions. The endpoint was defined as the last worker serum dilution having an optical density greater than twice the mean of the negative control sera. These samples were also assayed for activity against HSA by an ELISA technique identical to that described above except that the first addition to the microtiter well was HSA instead of m-TMXDI-HSA or m-TMI-HSA. The assays were performed by each of two individuals who had no knowledge of the questionnaire data. Any discrepant results were repeated. If a serum had similar activity against HSA and m-TMXDI-HSA or m-TMI-HSA, the isocyanate antibody was considered to be negative.

Control sera

Negative control sera were obtained from non-exposed asymptomatic individuals. Serum from an individual with positive antibody titers to HDI-HSA was used as a positive control serum.

Final Evaluation

'I'he final evaluation of these workers' respiratory and ocular symptomatology was based on both serologic results and questionnaire assessment.

Results

Exposure to m-TMXDI

During routine conditions, personal sampling measurements of m-TMXDI in 1984, 1985, and 1988 ranged from < 0.0004 to 0.0102 ppm. Sixty-five people worked in jobs with this range of exposures. The remaining workers were exposed to less than 0.0004 ppm. During the start-up phase of commercial production in 1987 and 1988, potential exposure to m-TMXDI is estimated to have been greater due to spills resulting from process upsets and discontinued process operations. Workplace air concentrations were estimated for these activities to range from 0.3 to 0.5 ppm.

Questionnaire Assessment

The results of the clinical assessment of the workers' ocular and respiratory symptoms are listed in Table 1.

Table 1: Workers' questionnaire assessment, serologic results, and final evaluation, reproduced from (Grammer et al., 1993)

Group	Total	Irritant symptoms (%)	Positive antibody (%)	NIRDRTT* (%)
1[$]	31	14 (48)	0 (0)	31 (100)
2[&]	65	25 (39)	8 (12)	65 (100)

* "No Immunologic Respiratory Disease Related To m-TMXDI or TMI"; [$] Exposure < 0.4 ppb; [&] Exposure < 0.4 – 10.2 ppb

Thirty-nine workers had symptoms consistent with an irritant syndrome. Eleven workers had only irritant eye symptoms while three had only irritant throat symptoms. No worker had symptoms suggestive of new onset asthma.

TMXDI-HSA and TMI-HSA conjugates

The m-TMXDI-HSA and m-TMI-HSA conjugates demonstrated altered electrophoretic mobility when compared with sham-conjugated HSA. The epitope density of the m-TMI-HSA was 11 and that of the m-TMXDI-HSA was 29.

ELISA

One worker had low-level IgE (1:10) against TMXDI-HSA; that worker reported no work-related respiratory symptoms. Seven workers had low-level IgG (1:10) against m-TMXDI-HSA; two of those seven workers also had very low-level (1:10) IgG against TMI-HSA. All eight of the workers with antibody worked in jobs with the higher exposure range (cf. Table 1).

Final Evaluation and discussion

In the 96 workers exposed to m-TMXDI and m-TMI, there were 39 workers with irritant symptoms, mostly upper respiratory symptoms. There were eight workers with antibody against m-TMI-HSA or m-TMXDI-HSA; only one of these had specific IgE, and that worker had no work-related symptoms. There were no workers who reported new onset asthma symptoms, but two workers reported exacerbation of pre-existing asthma with isocyanate exposure. In short, there was a low incidence of positive serology and no clinical hypersensitivity disease.

The authors concluded that none of the 96 workers had an immunologically mediated respiratory or ocular disease from exposure to m-TMI or m-TMXDI, nor did non-immunologic sensitisation appear to occur, as none of the workers reported histories compatible with new onset symptoms (Grammer et al., 1993).

1.1.2 **Human data for the category source substances HDI, MDI, TDI**

1.1.2.1 Case reports

Table 2: Cases related to HDI, MDI, and/or TDI as documented in the published literature (non-comprehensive)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of three painters with respiratory tract symptoms	#1: Spray-painting with polyisocyanate lacquer #2: Painting with polyisocyanate plastic lacquer #3: Spray-painting, brush-painting with plastic lacquer	TDI	#1: Asthmatic bronchitis #2: Asthmatic symptoms/attacks #3: Not specified (severe cough, pressure on the chest)	(Swensson et al., 1955)
Case report of six subjects with respiratory symptoms suggestive of diisocyanate sensitisation	Developmental and experimental work on urethane foams and surface coatings; #1: Engineer, known to be sensitised to TDI. Re-exposure occurred unintentionally due to an accident. #2/3/4: Laboratory assistants using TDI to produce plastic foams. #5: Fitter dismantling equipment which was used in the making of foam. #6: Not accepted as a case of sensitisation as symptoms were attributed to anxiety.	TDI	TDI respiratory sensitisation as demonstrated by respiratory symptoms	(Williamson, 1965)
Examination by bronchial provocation test for sensitivity to TDI of 24 workers with respiratory disease handling diisocyanates	Not specified	HDI. MDI, TDI	Asthma	(O'Brien et al., 1979)
Study to determine the mechanisms of bronchial hyperreactivity ("sensitivity") to TDI in 28 workers with a history of sensitivity to TDI	TDI production	TDI	Asthmatic reactions; five workers were identified as non-reactors	(Butcher et al., 1979)
Case report of two workers with respiratory symptoms	Not specified #1: Production supervisor #2: Welder, exposed continuously to polyurethane foam fumes	MDI	#1: Occupational asthma #2.: Hypersensitivity pneumonitis	(Zeiss et al., 1980)
Radioallergosorbent testing of 26 TDI-reactive individuals shown to react to provocative inhalation challenge with TDI	Not specified	TDI	Asthma	(Butcher et al., 1980)
Case report of four subjects diagnosed with MDI-related asthma	Welding of polyurethane belts	MDI	Asthma	(Lob and Boillat, 1981)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of subject with repeated prolonged exposure to MDI	Manufacturing engineer	MDI	Hypersensitivity pneumonitis and pleuritis progressing to fibrosing alveolitis	(Friedman, 1982)
Inhalation challenge tests in exposed workers with respiratory symptoms related to TDI or MDI	MDI: Not specified; TDI: Printers and laminators of flexible packaging	TDI, MDI	Occupational asthma in 24/40 workers with MDI- and 30/51 workers with TDI-related respiratory symptoms	(Burge, 1982)
Case report of subject with history of shortness of breath, wheezing, malaise and chills	Foreman in a garage where painting was done using a polyisocyanate activator	HDI	Combined alveolitis and asthma	(Malo et al., 1983)
Retrospective analysis of 109 MDI production workers	MDI production	MDI	8/109 workers were diagnosed with chronic obstructive bronchial disease and 3/109 with contact dermatitis.	(Diller and Herbert, 1983)
Case report of one subject	Manufacture of shoe soles	MDI	Occupational asthma	(Innocenti and Paggiaro, 1983)
Case report of one patient with symptoms of hypersensitivity pneumonitis	Packing and shipping of automobile equipment, occasionally engaged in spraying a mixture of MDI and polyol to produce polyurethane foam	MDI	Hypersensitivity pneumonitis	(Baur et al., 1984)
Case report of one patient showing symptoms of severe asthma	Grain elevator operator/repairman cutting polyurethane plate made of MDI	MDI	Occupational asthma	(Chang and Karol, 1984)
Case report of two patients with developed asthma and/or alveolitis	Painting, insulating	HDI, MDI	Asthma, alveolitis	(Laitinen et al., 1984)
Mechanistic challenge study in four subjects exhibiting a late asthmatic response after TDI exposure	Not specified	TDI	Asthma	(Mapp et al., 1985)
Case-control study in 78 workers with respiratory symptoms,.372 railway yard repair workers, representing 95 % of the work force, served as negative controls.	Iron and steel foundry; workers handling PepSet, a chemical binding system containing MDI	MDI	Asthma (12/78)	(Johnson et al., 1985)
Case report of two workers who developed asthmatic symptoms	Gym-shoe factory, injecting MDI into shoe soles	MDI	#1: Asthma, hypersensitivity pneumonitis #2: Asthma	(Mapp et al., 1985)
Case report of one patient with a history of respiratory illness	Chemical industry technical representative, exposed while unloading a railroad tank car containing MDI and having further work-related intermittent exposure	MDI	Occupational asthma	(Banks et al., 1986)
Case report of one patient with asthma persisting for twelve years after single massive exposure to TDI	Not specified	TDI	Asthma	(Moller et al., 1986)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of four workers with respiratory symptoms	Iron foundry; core making, sand mixing, and fettling associated with the Cold-Box process	MDI	Asthma bronchiale due to contact with isocyanates	(Erban, 1987; Erban, 1988).
Study on the inhibitive effect of prednisone on late asthmatic reactions and airway inflammation induced by TDI in eight sensitised subjects with previously documented late asthmatic reactions	Not specified	TDI	Asthmatic reactions	(Boschetto et al., 1987)
Case report of one patient having TDI-induced asthma	Accidental peak exposure during maintenance work in a chemical plant (this peak exposure lead to onset of symptoms of asthma)	TDI	Isocyanate induced Asthma. Positive in 1974 (after accident), no hyperresponsiveness to challenge testing in 1985 (after 11 years without exposure to TDI), but positive in 1987 (after return to work with TDI).	(Banks and Rando, 1988)
Case report of one patient diagnosed with asthma induced by TDI	Self-employed car painter	TDI	Death after an asthma attack The subject was recommended to cease working with isocyanates after diagnosis of asthma induced by TDI in 1980. Nevertheless he continued under usage of anti-asthmatic drugs. He died 1986 within 1 hour of the second exposure to a new kind of polyurethane paint in the workplace.	(Fabbri et al., 1988)
Challenge study examining cross-reaction between TDI and MDI in 25 subjects having developed asthma to TDI	Furniture industry, handling polyurethane varnishes catalysed with TDI	TDI	Occupational asthma	(Innocenti et al., 1988)
Case report of eight patients with an unequivocal history of professional asthma	#1: Employee in polyurethane foam car seat manufacture #2, 4, 5, 6, 7, 8: Workers in shoemaking factory #3: Shoemaker	HDI, MDI., TDI	Occupational asthma	(Cvitanovic et al., 1989)
Assessment of specific IgE and IgG antibodies in 62 workers with possible occupational asthma caused by isocyanates	Workers in foam industry (TDI), spray painters (HDI/MDI), various (MDI)	HDI, MDI, TDI	Occupational asthma; specific inhalation challenges were positive in 29 subjects.	(Cartier et al., 1989)
Case report of two subjects showing respiratory symptoms	Not specified	MDI	Occupational asthma	(Malo et al., 1989)
Group-based report on 63 workers with a diagnosis of probable isocyanate-induced asthma	Manufacture of TDI, manufacture of foam, manufacture of refrigerators	TDI	TDI-induced asthma in 30/63 workers	(Banks et al., 1989)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of one subject complaining of nocturnal dyspnoea and dry cough	Paint processing plant	TDI	Hypersensitivity pneumonitis due to isocyanates	(Nozawa et al., 1989)
Case report of one patient with symptoms of non-cardiac chest pain probably secondary to pleuritis	Worker manufacturing award placques with a polyurethane coating resin containing MDI	MDI	Isocyanate-induced asthma	(Sales and Kennedy, 1990) and
Case report of six workers with respiratory complaints	Production of polyurethane foam; #1, 2, 3, 5: Workers manufacturing polyurethane foam #4: Research technician #6: Worker in the shipping department; Later all six worked in areas with negligible/no exposure to TDI	TDI	TDI-induced occupational asthma	(Banks et al., 1990)
Case report of 13 workers with respiratory symptoms consistent with asthma	Manufacture of waferboards; workers performing routine (i.e. waxing of former conveyor belt) and non-routine (unplugging jammed conveyors, repairs, adjustments) maintenance tasks	MDI	Occupational asthma (12 cases) and hypersensitivity pneumonitis (1 case)	(Reh and Lushniak, 1984)
Case report of one patient with, *inter alia*, bilateral pleuritic chest pain and haemoptysis	Spray-painter spraying isocyanate-containing paint onto warm metal	HDI, another isocyanate (possibly TDI)	Haemorrhagic pneumonitis	(Patterson et al., 1990)
Evaluation of the morphologic basis of the different outcomes of TDI asthma after quitting occupational exposure in ten patients with TDI asthma	Not specified	TDI	Asthma	(Paggiaro et al., 1990)
Case report of one patient having bronchospasms after burning polyurethane packs and an immediate asthmatic reaction while working with polyurethane foam.	Task at work:Burning polyurethane packs Task at home: Insulating a window/drilling dry polyurethane foam Tasks with unspecified location: Painting cars with isocyanate-containing paints	MDI, TDI	Immediate bronchial hyperreactivity	(Dietemann-Molard et al., 1991)
Study reassessing temporal patterns of bronchial obstruction after exposure to diisocyanates in 23 subjects that were referred for investigation of occupational asthma and underwent specific inhalation challenges with positive results	Six foam industry workers, ten spray painters, seven employees from various industries (plastics, foundries)	HDI, MDI, TDI	Occupational asthma	(Perrin et al., 1991)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Study of blood parameters in ten subjects, previously shown to develop a dual or late asthmatic reaction after inhaling TDI	Not specified	TDI	Occupational asthma	(Finotto et al., 1991)
Evaluation of 23 employees complaining about work-related respiratory symptoms	Paint mixers and spray-painters	TDI	Asthma in 3/23 patients	(Park et al., 1992)
Case report of two workers with asthma	Wood-roof maintenance workers brushing/rolling lacquers/varnishes containing TDI	TDI	Occupational asthma	(Vandenplas et al., 1992a)
Case-control study of activated T-lymphocytes and eosinophils in the bronchial mucosa of patients with isocyanate-induced asthma; nine occupationally sensitised subjects and twelve healthy non-atopic control subjects were tested.	Not specified	MDI, TDI	Occupational asthma	(Bentley et al., 1992)
Case study of a man with dry cough and exertional dyspnoea	Handling spray-paint containing isocyanates	TDI, MDI	Hypersensitivity pneumonitis	(Akimoto et al., 1992)
Cross-sectional study in 216 coal-miners exposed to MDI showing symptoms of work-related shortness to breath	Coal miners working in rock consolidation with MDI	MDI	Specific bronchial hyperresponsiveness to MDI (4), isocyanate asthma (2)	(Lenaerts-Langanke, 1992)
Evaluation of closed-circuit methodology for inhalation challenge test with isocyanates in 20 consecutive workers suspected of having isocyanate-induced asthma	Not specified	HDI, MDI, TDI	Occupational asthma in 6/20 workers	(Vandenplas et al., 1992b)
Specific inhalation challenge study in workers with possible occupational asthma	Not specified Workers exposed to spray paints	HDI	Occupational asthma in 10/20 workers	(Vandenplas et al., 1993a)
Inhalation challenge study in workers complaining of respiratory and general symptoms related to workplace exposure	Manufacture of woodboard chips with MDI-based resin #1: Maintenance mechanic #2: Production line welder #3: Quality control laboratory #4: Electrician #5: Industrial mechanic #6: Production supervisor #7: Cleaning #8: Casual	MDI	Hypersensitivity pneumonitis	(Vandenplas et al., 1993b)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Examination of seven subjects with occupational asthma induced by TDI or MDI and three control subjects never exposed to isocyanates	Not specified	MDI, TDI	Occupational asthma	(Calcagni et al., 1993)
Patient claiming compensation for bronchial asthma	Surface worker in a coal mine involved in polyurethane rock consolidation	MDI	Occupational asthma	(Nemery and Lenaerts, 1993)
Case-control study of sputum eosinophilia after asthmatic responses induced by isocyanates in 9 subjects with occupational asthma induced by MDI or TDI and four control subjects	Not specified	MDI, TDI	Occupational asthma	(Maestrelli et al., 1994a)
Study examining CD8 T-cell clones in bronchial mucosa of two patients with asthma induced by TDI	Use of polyurethane paint	TDI	Occupational asthma	(Maestrelli et al., 1994b)
Case report of 14 patients suspected of isocyanate-induced hypersensitivity pneumonitis.	#1, 3, 10, 12, 14: Foam production #2, 8, 9: Paint spraying (#4: Plastic welding) #5, 11: Adhesive application #6, 7, 13: Injection molding	HDI. MDI, TDI, HDI, (TDA/TIPHP in #4)	Hypersensitivity pneumonitis	(Baur, 1995)
Study on the outcome of specific bronchial responsiveness to occupational agents after removal from exposure in 15 subjects with occupational asthma	Not specified	HDI, MDI, TDI	Occupational asthma	(Lemière et al., 1996)
Case report of one subject with occupational asthma	Steel foundry; mold and core processing with use of resins containing MDI	MDI	Occupational asthma (1986) followed by fatal asthma attack (1992)	(Carino et al., 1997)
Case report of one subject with breathing difficulties	Carpenter/glueing wood onto aluminium sheets	MDI	Asthma and contact urticaria	(Kanerva et al., 1999)
Inhalation challenge study in 24 symptomatic subjects	Not specified	HDI, MDI, TDI	Occupational asthma	(Malo et al., 1999)
Analysis of specifig IgG response to isocyanates in 13 subjects with respiratory reactions	Not specified	HDI. MDI, TDI	Occupational asthma (12), hypersensitivity pneumonitis (1)	(Aul et al., 1999)
Case report of one worker with respiratory symptoms, who was exposed for three years without developing sensitisation. Probably a single high dose after an accidental spill represented the trigger for sensitisation	Toy manufacture; spray painter/spray painting of polyurethance foam balls with a paint containing MDI	MDI	Occupational asthma	(Perfetti et al., 2003)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of a woman with breathing difficultie; symptoms started after a peak exposure (heavy and prolonged contact with a glue).	Manufacture of plastic components for the car industry using a two-component polyurethane glue	MDI	Occupational sensitisation to MDI causing contact urticaria and asthma simultaneously	(Valks et al., 2003)
Case report of one man complaining about respiratory symptoms	Handling of spray-paint containing isocyanate	MDI	Combined hypersensitivity pneumonitis and bronchial asthma	(Matsushima et al., 2003)
Case report of one patient with respiratory symptoms	Hospital nurse working with MDI-containing synthetic plaster casts	MDI	Occupational asthma	(Donnelly et al., 2004)
Case report of one man who reported coughing and fever	Breaking up a large refrigerator containing MDI	MDI	Hypersensitivity pneumonitis with acute respiratory distress syndrome	(Morimatsu et al., 2004)
Re-examination of 25 subjects diagnosed with occupational asthma after long-term removal from exposure	Spray-painting using polyurethane varnishes	TDI	Occupational asthma; re-examination of subjects with occupational asthma after 58 ± 7 months following removal from exposure. Seven were still reactors, 18 had lost reactivity.	(Pisati et al., 2007)
Case report of one subject complaining of breathing difficulties	Mixing polyurethane glues for the manufacture of adhesives	MDI	Asthma and urticaria (concomitant type I and type IV sensitivities to MDI)	(Stingeni et al., 2008)
Follow-up study in 17 patients diagnosed with diisocyanate-induced asthma after cessation of exposure	Not specified	HDI, MDI, TDI	Diisocyanate-induced asthma	(Piirilä et al., 2008)
Case report of one patient with an acute respiratory event	Paint quality controller (laboratory)	HDI	Occupational extrinsic allergic alveolitis; life-threatening allergic reaction	(Bieler et al., 2011)

Table 3 shows the results from studies regarding the annual incidence of TDI-related occupational asthma cases as reviewed by (Ott, 2002).

Table 3: Data taken from Tables II and III in (Ott, 2002)

Study	Time period	Annual incidence of TDI-induced occupational asthma [%]	TDI concentration [ppb]	Exposure sampling
TDI production units				
(Adams, 1975)	1961 - 1970	5.6	1962 - 1964: 58-72 % of samples > 20 1965 - 1966: 4-21 % of samples > 20 1967 - 1970: 1-2 % of samples > 20	Area samples
(Porter et al., 1975)	1956 - 1959	1.6	1956 - 1957: 60 (mean area conc.)	Area samples
	1960 - 1969	0.8	1960 - 1969: steady decline in area conc.	
	1970 - 1974	0.3	1974: < 4 (mean area conc.)	
(Weill et al., 1981)	1973 - 1978	1.0	1.6 - 6.8 (TWA; range by job) (STC > 20, 5-11 % of time in moderate to high exposure jobs)	Area samples 1973-75 Personal samples 1975-78
(Ott et al., 2000)	1967 - 1979	1.8	3.4-10.1 (TWA; range by job)	Area samples 1967-75 Personal samples 1976-96
	1980 - 1996	0.7	0.3-2.7 (TWA; range by job) (STC > 20, 0.5-0.9 times/shift in moderate to high-exposure jobs)	
PU foam production facilities				
(Woodbury, 1956)	1954 - 1955	5	Multiple TDI spill episodes described in 18-month period	No sampling data
(Williamson, 1964)	1962 - 1963	> 2.7	Samples mostly < 20 (up to 200 detected during spills)	Area samples
(Bugler et al., 1991)	1981 - 1986	0.8	0.9-2.6 (TWA; range by job) 22 % of 8-h samples with short-term conc. > 20 and 10 % > 40	Personal samples
(Jones et al., 1992)	1982 - 1986	0.7	1.4-4.5 (TWA; range by job) (STC > 20, 3 % of time in production and 0.1 % of time in finishing jobs)	Personal samples

1.1.2.2 Longitudinal studies

The available longitudinal studies are summarised in Table 4.

Table 4: Longitudinal studies on occupational asthma related to exposure to HDI, MDI, and/or TDI

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Adams, 1975)	Prospective cohort study (nine years), two plants 565 subjects employed for some period between 1961 to 1972 A) Comparison of respiratory symptoms in TDI plant workers (n = 76) with control workers (n = 76) in another plant B) Lung function in healthy workers (n = 180) C) Long-term effects in men removed due to symptoms without exposure to TDI since two to 11 years (n = 46) compared to age-matched control group (n = 46) D) Lung function in men removed due to symptoms and without exposure to TDI since two to 11 years (n = 61)	TDI Manufacture	Area samples taken at points in the plant where free TDI might have been expected (ca. 250 measurements a week; Marcali method, (Marcali, 1957)) Samples > 20 ppb: 1962-64: 58–72 % 1965-66: 4–21 % 1967-70: 1-2 %	A) **Respiratory symptoms** (questionnaire): No significant difference in symptoms between men working in TDI plant and controls, with the exception of higher frequency of wheezing in controls. B) **Lung function**: Duration of exposure had no effect on FEV_1 or FVC in the regression analysis. C) **Respiratory symptoms** (questionnaire): Prevalence of symptoms in TDI-sensitised men significantly higher than in controls → persistence of symptoms D) **Lung function**: FEV_1 and FVC smaller than predicted by equation obtained from a control group: FEV_1 -267 mL, FVC -269 mL	Reviewed in (Ott, 2002) Method of analysis did not calculate individual decline in lung function. Regression analysis included duration of exposure, but no exposure level Area measurements Lung function measurements in the afternoon Only healthy workers included Smoking not included in regression analysis

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Butcher et al., 1977)	Prospective cohort, 2.5 years Visits: April 1973 (before TDI production), November 1973 (after production had started), every six months thereafter Initially n = 166 Study in TDI-sensitive persons (specific and unspecific challenge)	TDI Manufacture	Area sampling (1973): frequent excursions of 8h-TWA value of 5 ppb; many above 20 ppb Personal monitoring (1975) Frequent and large discrep-ancies between simultaneously measured area and personal exposure levels Four groups: 1) Mainly in TDI area: n = 77 2) Intermittently in TDI area: n = 36 3) Comparison group: n = 53 4) Workers transferred from control group to exposure group after production had begun (added later)	**Lung function** changes (n = 102): Mean values of FVC and FEV_1 increased in all groups. Other lung function parameters decreased slightly (n. s. different from zero or predicted). Paradoxical differences for lung volumes and diffusion capacity (greater declines in the groups with higher exposure). No exposure-related excess decline in lung function determined. **Respiratory symptoms** (questionnaire administered by interviewers): No significant increase in prevalence of bronchitis, atopic disorders, upper respiratory symptoms from April 1973 to October 1975. Significant proportion of exposed workers (26 of 89) reported onset of lower respiratory symptoms after beginning work in TDI areas (due to symptom development in non-smokers). **Inhalation challenge** with TDI: Nine out of 13 workers had an adverse bronchial response (immediate type, late type or dual type). Some reacted at 5 ppb, some to a higher concentration only.	Attrition rate = 7.2 % Two workers had left the study by October 1975 after developing reactivity to TDI. No quantitative exposure es-timation for the four exposure categories Smoking not considered in analysis of change in lung function

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wegman et al., 1977)	Follow-up of (Wegman et al., 1974) 1972: n = 112 1974: n = 63 (available for re-survey); n = 57 with personal exposure levels	TDI PU cushion manufacture	118 area samples + 14 personal samples taken during study period to characterise 20 work stations Marcali method (Marcali, 1957) Each individual was classed according to his or her usual work station Three exposure groups (ppm): ≤ 0.0015 (n = 20) 0.0020 – 0.0030 (n = 17) ≥ 0.0035 (n = 20)	**Lung function** (because of acute effect seen on Monday: Monday morning following three-day weekend): Dose-response relationship for two-year change in FEV_1 (-12/-85/-205 mL from low to high exposure groups). Only those in lowest exposure group showed normal declines in FEV_1. Those in highest group had three- to fourfold higher FEV_1 declines than expected (103 mL/year). Significant association between acute and chronic decrement in FEV_1. **Respiratory symptoms** (questionnaire): Prevalence of cough and phlegm increased with increase in exposure. Wheezing and dyspnea not associated with exposure.	High attrition rate Followed up: (Wegman et al., 1982) Possible confounding variables explored: Age, months employed, smoking habits, variables related to lung size. Authors report that none of those was able to explain the differences.
(Diem et al., 1982)	Five-year prospective (9 surveys) First survey in 1973 (5 months before start of production) Initially: n = 168 After 5 surveys: n = 274 (males) Median follow-up time for n = 223 men who met inclusion criteria of spirometric data 4.1 years (1 – 5.5)	TDI manufacture	2093 personal samples from 143 workers representing all job categories 8 h TWA from 0.1 ppb - 25 ppb, geometric mean 2.00 ppb Average exposure: Three TWA exposure job categories: Geometric mean in ppb (time per shift < 20 ppb): Low: 0.02 (1.3 min) Medium: 2.0 (8.6 min) High: 4.5 (28.2 min)	**Lung function** (spirometry, annual change): Decrease in FEV, % FEV and FEF_{25-75} was significantly larger in the high cumulative exposure category than in the low category (adjusted for pack-years of smoking). No association of the other lung function annual changes with exposure. A more detailed analysis of FEV_1 and FEF_{25-75} in six categories of cumulative TDI exposure and smoking showed a significant effect of TDI exposure in never smokers only and a significant	No unexposed group "The present data do not identify a specific exposure below which no effect upon FEV_1 annul decline will occur. However, they do suggest that the NIOSH-recommended standard of a 5 ppb 8-h time-weighted average and a 20 ppb 10-min short-term exposure limit is reasonable."

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Diem et al., 1982), ctd.			Cumulative exposure calculated from number of months spent in each of the three TWA exposure categories and their respective geometric means. Workers were divided into two groups using a division point of 68.2 ppb-months (= 1.1 ppb x 62 months). Low exposure group n = 149, high n = 74. Working time spent > 5 ppb: 2 % in low exposure group, 15 % in high exposure group. Peak exposure categories: Division point 0.19 months > 20 ppb	effect of smoking in the low exposure group only. → effects not additive Effects similar for six categories of TDI peak exposure and smoking with the exception that a significant exposure effect was also found in current smokers → higher TDI exposure seems to mask smoking effect → peak exposure analysis suggests additive effect (lacking in cumulative exposure analysis) **Respiratory symptoms** (questionnaire): No significant correlation in increase in prevalence from initial to final interview and exposure to TDI.	Low cumulative exposure group was older and initially had higher prevalence of respiratory symptoms than high exposure group → possible underestimation of excess decline in lung function due to TDI 75 % of the low exposure group had follow-up time > 2.5 years and 99 % of the higher exposure group Atopy, race and smoking were considered Age and FEV_1 level were considered in the more detailed analysis of FEV_1 and FEF_{25-75}
(Musk et al., 1982)	Five-year follow-up n = 259 from three sites were examined in 1971; one of the sites closed in 1972 and there was high worker turnover; 107 subjects were available for re-examination in 1976	MDI and TDI for the manu-facture of PU automobile components	2573 environmental samples were collected by plant personnel in the breathing zone of subjects pouring urethane plastic (exposure in areas with the highest exposures were measured) During lung function survey further measurements were made by plant personnel and study personnel at selected sites with highest TDI and MDI concentrations Marcali method (Marcali, 1957)	**Lung function** (spirometry (FEV_1, FVC); change over 5 years/change over the course of a day/change between before and after two weeks of vacation): Mean annual decrement in FEV_1 of 0.02 L was interpreted as being only age-related No significant acute change in FEV_1 over the course of a day before or after vacation reported After two weeks of vacation FEV_1 was increased in those who had taken the vacation (n = 49, n. s.) and was decreased in those who had worked (n = 31, n.s.).	Uncertainties in exposure assessment and spirometry Smoking, age, height, sex were considered in the regression analysis of FEV_1. Healthy worker survivor effect (although it is reported that subjects who left had similar lung functions to the remaining subjects, it seems possible that workers left due to earlier symptoms of sensitisation).

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Musk et al., 1982), ctd.			All environmental measurements made over the 5 years together with the occupational history of the subjects determined the exposure category (No exposure/MDI/TDI/MDI and TDI). 90 % of all measurements of TDI taken over the four years prior to the follow-up study were < 5 ppb (plant 1) and < 4 ppb (plant 2) Geometric mean TDI concentration: 1.5 ppb (plant 1) and 1 ppb (plant 2) MDI levels tended to be lower than TDI levels	Exposure category did not affect daily change in FEV_1/pre- to postvacation change in FEV_1/five-year change in FEV_1. **Respiratory symptoms** (questionnaire): No association between exposure to isocyanates and bronchitis or dyspnea found No acute exposure-related symptoms reported	
(Wegman et al., 1982)	Four-year follow up (Wegman et al., 1974; Wegman et al., 1977) 1972: n = 111 1974: n = 63 1976: n = 48 (all those who were still at work in 1976) → n = 37 with exposure history and acceptable spirograms On all three occasions workers were examined before work and as many as possible six to ten hours later.	TDI Automobile seat cushion manufacture	Environmental sampling at selected work sites on the same day as lung function was measured. Additional sampling during the first two years of the study. Personal sampling in production area, area samples in warehouse and nonproduction sites. Marcali method (Marcali, 1957) Occupational histories taken from personnel records	**Lung function**: Acute change in FEV_1 (during work shift) observed at the beginning of the study was weakly associated with long-term change in FEV_1. Chronic change in FEV_1 (over four years): Mean exposure to TDI was the best predictor of four-year change in FEV_1 in a stepwise regression model. Change in FEV_1 increased with exposure and was significantly different between the exposure groups.	Uncertainties in exposure assessment High attrition rate Lung function decline evaluated from 3 occasions only

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wegman et al., 1982), ctd.			Cumulative exposure of each worker calculated and from this the usual exposure level. Three exposure groups: Low (< 2.0 ppb) Medium (2.0-3.4 ppb) High (> 3.5 ppb)	Decline in FEV_1 in high exposure group (60 mL/year) was higher than annual decline observed in other studies of normal populations (32-47 mL). **Respiratory symptoms** (questionnaire; upper respiratory tract symptoms: sneezing, sinus trouble or postnasal drip, hay fever; lower respiratory tract symptoms: coughing, wheezing, shortness of breath): Prevalence of respiratory symptoms was unrelated to exposure category.	
(Omae, 1984)	Two-year follow up Four TDI-producing plants, two research laboratories 1980: n = 106 male exposed workers n = 39 male controls (office workers) 1982 (one plant had closed): n = 64 workers (follow-up rate 60 %) n = 21 controls (follow-up rate 62 %)	TDI Manufacture; research laboratory	Mean duration of TDI exposure: 9.0 years (subjects in 1980) 11.2 years (subjects in 1982) Personal paper tape monitor (gives continuous profile; n = 161 samples in 1980, 106 in 1982) Means of individual TWA: 0.7 ppb (1980) 1 ppb (1982) Short-term exposure ≥ 20 ppb in 9.3 % (1980) and 1.9 % (1982) of collected samples	**Lung function** (Maximum expiratory flow volume curve, respiratory impedance): Eight workers with asthmatic reactions, shortly after having begun work with TDI. Percentage of predicted values significantly less than 100 % in some of the expiratory flow parameters. No significant differences in lung function between the exposed workers and the referents. Change in lung function over the day (1980; 68 TDI workers + 31 controls): No meaningful daily changes in lung function in either group. Change in lung function over two years: When adjusted for aging, no remarkable intra-individual two-year decreeses in lung function parameters in both groups and no significant difference between the groups.	High loss to follow-up Co-exposures: TDI plant workers: occasionally various irritants such as phosgene, chlorine, nitric acid, sulfuric acid; Research laboratory workers: irritative amines, organic tin compounds , MDI, HDI during experimental mold foaming Effects of age, physical factors and smoking on lung function considered in analysis Survival worker effect considered to be small by the authors Hyperreactive persons to TDI may have already been transferred out of TDI sections

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Omae, 1984), ctd.				No difference in the two-year decrement between the workers with asthmatic reactions and the other TDI workers. **Symptoms** (interviewed by means of a questionnaire): No significant differences in prevalence of respiratory symptoms between exposed workers and reference. Significantly higher prevalence of throat and eye irritation in exposed workers than in reference. May be due to peak exposures to TDI or other irritants (phosgene).	
(Gee and Morgan, 1985)	Ten-year follow-up (includes significant proportion of subjects included in (Musk et al., 1982)Musk et al. 1982) Examinations in 1971 and in 1981 n = 68 exposed n = 12 controls n = 65 subjects with pre- and post-shift measurement n = 42 studied in 1971 and 1981	MDI and TDI Manufacture of fittings, seat covers, other fixtures used in the interior of cars	Routine area and some individual sampling had been carried out monthly or more frequently Mean annual concentrations between 1973 and 1980 for TDI: 1- 5 ppb Mean annual concentrations between 1975 and 1981 for MDI: 1- 5 ppb	**Lung function** (compared to predicted values): Three subjects had impaired lung function (two exposed, one control). Lung function of subjects studied previously had mean FVC and mean FEV_1 > 100 % of the predicted values. Control group of one plant had a significantly lower percentage of the predicted FVC and FEV_1 than the exposed group. No other significant difference between any of the groups. Lung function (change over shift): Change not higher than 10 % in any subject. No comparison between controls and exposed.	Mean annual exposure values on factory level only Uncertainties in spirometry data (no reproducibility, leak in spirometer possible in 1971; learning effect from pre- to post-shift measurements) Results on annual decline in lung function seen as “not realistic” (small increase in FVC, small decrease in FEV_1).

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Gee and Morgan, 1985), ctd.				Mean shift change in FEV_1 was -57 mL in exposed and +69 mL in controls in one plant and -23 and -80 mL in the other plant, respectively.	
(Musk et al., 1985)	Re-analysis of the data from (Musk et al., 1982)				The spirograms performed 1971 in the study by (Musk et al., 1982) were criticised ("inadequate", "lack of reproducibility", "leak in the spirometer"). (Musk et al., 1985) found the original conclusions valid.
(Pham et al., 1988)	Five-year follow up 1976: n = 318 workers (104 women) 1981: n = 156 (45 women) Two factories producing PU foam Follow up of Pham et al. 1978	Mainly MDI Production of PU foam	Isocyanate concentration: 1976: < 20 ppb 1981: ≤ 5 ppb 1976: Group I (n = 83): unexposed Group II (n = 117): indirectly exposed Group III (n = 118) directly exposed 1981: Only results for men reported for the longitudinal analysis. Group A (n = 45): unexposed at both studies Group B (n = 24): undirectly exposed at both studies Group C (n = 30): directly exposed at both studies Group D (n = 15): exposed in 1976, but removed in 1981	**Lung function** (flow volume curve, single breath CO diffusion test (D_{LCO})): Ventilatory function and lung transfer factors significantly impaired in male exposed workers compared to group I. Only in the subgroup of workers exposed for more than 5 years. Decline of ventilatory function variables not significantly different between the groups. Significant larger loss of D_{LCO} in subjects with persisting exposure (group C) compared to reference group. Results returned to normal for the subjects no longer exposed (group D). **Respiratory symptoms** (question-naire): Increased prevalence of asthma in group II men and group III women and of chronic bronchitis in both sexes. Number of workers with asthma or chronic bronchitis increased over the five years, but this was not limited to the exposed group.	High loss to follow up (only half of the initial cohort still active after 5 years) Rare information on exposure In females, the proportion of smokers was the same in groups I – II. In males, there were slightly (n.s.) more smokers in groups II and III. Co-exposure to other isocyanates? ("mainly MDI")

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Tornling et al., 1990)	Six-year follow-up (initial study: (Alexandersson et al., 1987)) 1978: 46 male car painters and 142 male controls (car platers and mechanics) randomly chosen from 14 garages in Stockholm Reinvestigation in 1984: Participation rate 78 % for car painters and 81 % for controls n = 36 car painters n = 115 controls	HDI monomer (and HDI biuret trimer) Car painting	Individual exposure assessments by industrial hygienist (interview about working routines, respirator use, hygienic standards). Exposure measurements at seven representative shops 98 samples inside and outside the respirator Individual exposure was calculated from workplace data, proportion of work tasks, use of respirators. 18 peak exposure measurements (sampling time < 3 min) Calculated TWA exposure: HDI: 0.0015 mg/m^3 (HDI-BT: 0.09 mg/m^3, frequently peak exposures > 0.2 mg/m^3) Calculated yearly number of peak exposure situations up to 6000 for each car painter No close correlation between exposure peaks and mean exposure	Decline in **lung function** over six years (1978: Monday morning values were used; 1984: Workers were examined during the first three hours of a working day): Smoking and ex-smoking car painters had significantly larger lung function decrease compared with respective controls. Non-smoking car painters displayed no faster deterioration in lung function than corresponding controls. (Decrease in FVC correlated significantly with number of HDI-BT exposure peaks, but not with mean exposure.) **IgG and IgE**, specific IgE in car painters: No significant differences in Ig levels between car painters and controls. No specific IgE found. **Symptoms**: Car painters reported significantly higher frequency of wheezing than the controls. Differences for other symptoms n.s.	Participation rate at follow-up 78 % among car painters and 81 % among controls. Selection bias (drop-outs may have quitted job because of respiratory symptoms, one asthma case known) Smoking not quantified

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Jones et al., 1992)	Cross-sectional, follow up Two plants n = 394 at the start of the study, through the fourth examination n = 435 had ever worked in one of the plants	TDI Production of flexible PU foam products	258 workers wore monitors on 507 shifts resulting in 4845 12-min samples: 9 % > 5ppb 1 % > 20 ppb TDI concentrations were assigned to groups of jobs. Information on the number of months spent in each exposure grouping was taken from personal records. Mean by plant and job area ranged from 1.17 to 4.47 ppb. Exposure measures: Cumulative exposure from hire to first study examination; cumulative exposure from hire to the end of study; cumulative exposure during the study period; length of time exposed to concentrations > 5 and 20 ppb	**Lung function** (spirometry, standing position, nose clips): Significant adverse effect of cumulative TDI exposure on initial level of FVC and FEV1 in current smokers. TDI exposure had no significant effect on lung function decline. **Respiratory symptoms** (questionnaire administered by trained interviewers): Chronic bronchitis more prevalent among those with higher cumulative exposure (controlled for smoking, age, sex). Metacholine challenge (n = 303): Metacholine responsiveness in 22 % of tested workers. Skin prick test with common inhalant allergens Total IgE, RAS	Co-exposure to different amines and other substances in foam production Healthy worker effect (predicted values) Differential misclassification of exposure (large number of samples < LOD)
(Omae et al., 1992)	Four-year follow up (cross-sectional results see (Omae et al., 1992)) Cross-sectional: 1981 Follow-up visits: 1983 and 1985 Japan:	TDI PU foam manufacture	Personal paper-tape monitors (n = 59 samples in 1981, 48 in 1983 and 52 in 1985) Group L (low exposure with little variation), n = 28, 17.4 years in the PU foam factories (mean), TWA (mean, max) 0.1 ppb, 1 ppb; Peak exposure level < 1 ppb Group H (exposed workers), n = 29, 16.5 years in the PU foam	**Lung function** (Flow-volume indices in 1981; Average annual loss of the indices during 1981-1985 (forced expiratory flow-volume test at follow-ups; slope of the regression equation for every subject)): No “noteworthy” differences in pulmonary function indices and average annual losses between groups H, L, reference.	No individual exposure estimates No significant differences between group H1 and H2 (as suggested in the abstract) Workers in slab-type factories intermittently exposed to relatively high levels of TDI and concurrent other chemical gases/aerosol → group H divided into two subgroups

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
	57 PU foam workers (follow-up rate 66 %; two excluded) 24 reference workers (follow-up rate 61 %; three excluded)		factories (mean), TWA (mean, max) 5.7 ppb, 30 ppb; Peak exposure level 3-80 ppb Two subgroups of group H: Group H1 (high short-term exposures), n = 15, 13.8 years in the PU foam factories (mean), TWA (mean, max) 8.2 ppb, 30 ppb; Peak exposure level 30-80 ppb Group H2, n = 14, 19.4 years in the PU foam factories (mean), TWA (mean, max) 1.7 ppb, 4 ppb; Peak exposure level 3-14 ppb	Group H1: Significantly larger average annual lung function losses (%MMF, $\%FEV_1$, $\%MEF_{25}$) than expected. Significantly larger average annual losses in some obstructive pulmonary function indices than in group L or reference group.	Smoking rate significantly lower in group H than in group L and reference group Comparison of average annual losses of smokers and non-smokers in the 4 groups showed similar trends. Higher losses in smokers than non-smokers. Based on a comparison between lung function of followed-up and lost workers, survival-worker effect was evaluated to be small.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Dahlqvist et al., 1995)	Re-analysis of data from (Tornling et al., 1990) and (Alexandersson et al., 1987) Evaluation if lung function decrease within the week is a marker of vulnerability of further decrement in lung function Six-year follow up, two study occasions Original group of workers were randomly chosen from 14 garages in Stockholm, 28 car painters participated in all three spirometric examinations, only those 20 were chosen who had been working during the entire six years period	HDI Monomer (and biuret trimer) Car painters working with polyurethane paints	Individual exposure assessments by industrial hygienist (interview about working routines, respirator use, hygienic standards). 81 exposure measurements for three tasks in 25 spray-painting chambers. Peak exposure measurements were performed (sampling time < 3 min) TWA between 1978 and 1984 for the workers studied: HDI: 0.0014 mg/m^3 (HDI-BT: 0.09 mg/m^3)	**Lung function** (1978: spirometry on Monday before work after two days of no exposure and on Friday; 1984: spirometry during the first three hours of a working day) Changes in FEV_1 and FVC within the week were dichotomised. Ten workers had a decrease in FVC within the week. Ten workers had a decrease in FEV_1 within the week. Car painters in the initial study who showed a decrease of FVC within the week in 1978 had a significantly greater decline in FVC from 1978 to 1984 than car painters who did not (adjusted for smoking). Significant correlation between changes within the week and six years decline in FVC. Decline in FVC was not significantly correlated with the mean exposure to HDI (or HDI-BT) estimated during the entire follow up. (Six year decline in FVC was correlated to the yearly number of peak exposures to HDI-BT.) **Respiratory symptoms** reported (for example 3/10 workers with change in FVC within the week in 1984 had cough, dyspnoea, and/or wheeze).	Uncertainties in exposure assessment (Current smokers on average had a higher yearly number of peak exposures to HDI-BT than the smokers as a whole (previous and current)..May indicate less use of protective equipment by smokers.) Smoking not quantified

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Akbar-Khanzadeh and Rivas, 1996)	1) Cross-sectional (daily, weekly changes) 2) Longitudinal (2.5-year follow up) 1) 16 Urethane mold operators 19 Controls (final assembly department, office area) 2) Oct 1989 – March 1992: 65 exposed to diisocyanates and solvents 40 exposed to solvents 68 controls (office, assembly, hardware department)	HDI monomer (and polyisocyan-ate), combined with organic solvents (MDI) Encapsulated automobile glass plant	1) HDI monomer, HDI polyiso-cyanate, volatile organic compounds Personal and area samples HDI: 92 % < LOD (set to 50 % of LOD); mean concentration (personal, area): 1.55 ppb (n = 6), 0.65 ppb (n = 3) (HDI polyisocyanate: 75 % < LOD; mean concentration (personal, area): 0.09 mg/m^3 (n = 6), 0.02 mg/m^3 (n = 3)) 2) Mean concentration: HDI 1 ppb (n = 8 samples) (HDI polyisocyanate 0.29 mg/m^3 (n = 5 samples)) MDI 0.45 ppb (n = 7 samples)	1) **Lung function** (spirometry on Monday and Friday before and after shift): No significant differences between exposed and control group No significant reduction in lung function during workshift or during week in the exposed group compared to the control group. Some findings in subgroups by sex. **Respiratory symptoms** (questionnaire): Some symptoms more prevalent in control group (n. s. or not tested?). 2) **Lung function** (spirometry before the shift): Significant decrease in lung function parameters in isocyanate/solvent-exposed group. Significant differences in lung function change (FEV1 and FVC) among groups **Respiratory symptoms** (questionnaire): Proportion of subjects who developed respiratory symptoms in the isocyanate-exposed group was not significantly greater than that of the non-exposed group.	No individual exposure estimates Very small number of air samples Control group appropriate? 1) HDI in control area 0.67 ppb Co-exposure Smoking was significantly more prevalent in the exposed group 2) Co-exposure Controls had no occupational exposure “between the two tests”

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Clark et al., 1998)	5 years longitudinal UK 780 workers in 12 factories (623 original + 157 naïve workers)	TDI Manufacture of PU foam	Personal monitoring (2294 measurements) for 100 job categories. Cumulative exposure between first and last lung function measurement was calculated for each subject based on job histories. 8 h TWA exposure limit of 5.8 ppb (46 ppbh for an 8 h working day) was exceeded on 107 (4.7 %) occasions. Five of the 780 subjects (0.6 %) had a mean daily exposure exceeding the limit value. Peak exposure limit value of 20 ppb was exceeded in 500 (19 %) samples. 8.8 % of the peak measurements > 40 ppb Exposed group (n = 521): Manufacture of PU foam or handling freshly manufactured products; mean daily exposure 9.6 ppbh (1.2 ppb 8 h TWA) Handling group (n =123): Handling cold PU products Low-exposure group (n =136): shop floor and office workers	Longitudinal decline in **lung function** (spirometry; three or more measurements): No significant effect of TDI on annual lung function change. For the naïve population, regression analysis showed a significant effect of mean daily exposure on annual changes of FEV_1 and FVC. Due to irritant effect? **Respiratory symptoms** (questionnaire): Increase in respiratory symptoms in exposed group and handling group, significant for wheezing. 24 cases of respiratory sensitisation were identified during the study.	Followed up by Clark et al. 2003 High attrition rate (47%) Leavers reported excess breathlessness and wheeze compared to non-leavers of the total population. Linear regression considered sex, group, age, age^2, smoking, mean daily exposure, peak exposure, pre-study exposure.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Hathaway et al., 1999)	Nine-year follow-up Production began in 1988, follow up through 1997 n = 43 "potential cases" and n = 42 "potential controls" of another unit at the same plant n = 32 matched pairs (by smoking, sex, age and by race and height if multiple possibilities were available)	HDI Production of HDI biuret and trimer from monomer	Average number of years of potential exposure: 8.4 Area and personal sampling (different methods and equipment over time) Exposure when not wearing respiratory protection was considered 1992-1995 (personal monitoring): average (range): TWA during work not requiring respiratory protection in the unit (1 – 4 hours/day): 0.5 ppb (0.0 – 2.0 ppb); calculated as 8h-TWA: 0.13 ppb Highest daily peak exposure: 2.9 ppb (1.0 – 10.0) Exposure before 1992 believed to be somewhat higher (no quantification)	**Lung function** (as part of annual evaluation of workers): Average number of available tests for calculating slope: 7.8 (exposed) and 8.2 (controls). No significant difference in annual change of lung function (slopes) between exposed and control group. By smoking status, the results show more variation. Results seen as being within the range of lung function declines reported in other studies.	Exposure not measured on individual level Smoking not quantified Height and race only partially controlled Co-exposure in control group reported (depending on work area): cerium and neodymium oxides, nitric acid, ammonia, kerosene, tributyl phosphate Qualitative information on potential drop outs: low turnover rate, few transfers between the units, subject attrition not been a problem
(Ott et al., 2000)	Historic cohort study using medical records and exposure records from 1967 to 1997 313 employees ever assigned to the TDI production unit for ≥ 3 months; 158 reference employees;	TDI manufacturing	Duration of TDI unit assignments: 5.7 years (average, men) 4.7 years (average, women) 3 months to 30 years (range) 1967 (area sampling): < 10 ppb in most areas and 25 ppb in the residue handling area	**Occupational asthma**: Case identification was based on site physician. One episode of asthma-like symptoms was not enough to be an OA case. 19 asthma cases presumed to be due to TDI, 9 skin allergies, 1 case of asthma and skin disease; Yearly incidence: 19 cases in 1779 work years = 1.1 %; before 1980: 1.8 %; since 1980: 0.7 %	Long follow-up time Exposure concentration linked to the asthma incidence not clear. (Ott et al., 2003) report for this study an exposure of 0.3 – 2.7 ppb (TWA; range by job) since 1980, assigning this to a yearly incidence of 0.7%.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Ott et al., 2000), ctd.	40 records were not found (16 of the study group and 24 of the reference group)		1969-1973: < 10 ppb in most areas with 60 to 80 ppb in certain areas 1976-1988 (personal 8 h samples, paper type method): 5.9 ppb (average) 1989-1997 (personal 8 h samples, filter method); 2.8 ppb (average) JEM: Industrial hygiene measurements were linked to job-specific work history per person; peak exposure and 8 h TWA concentration were aggregated on a job- and time-specific basis for three job groups (potentially low/moderate/high TDI exposure); cumulative dose estimates (ppb-months) Average TDI concentration: < 5 ppb for 59 % of the workers Cumulative TDI dose: < 500 ppb-months for 89 % of the workers Frequencies of peak exposure > 20 ppb per shift: 0.5 in moderate exposure jobs, 0.9 in high-exposure jobs	Cumulative incidence for people assigned to TDI unit for at least 20 yrs: 11.5 % (95 % CI 5.3-17.7 %) 7 of 19 cases had reported previous incidents of exposure to TDI (two related to rashes that had developed while handling TDI or waste products containing TDI) **Respiratory symptoms**: Since 1980 a standardised questionnaire was used that contained four questions with dichotomous answers (concerning wheezing/cough/chest discomfort/shortness of breath). No significant associations with responses in the questionnaires were found for those exposed to TDI versus referents. **Lung function** (spirometry): Neither cross-sectional nor longitudinal analyses of FVC and FEV_1 showed significant dose-response findings relative to exposure to TDI across the total exposed population.	Peak exposure and dermal exposure make it difficult to evaluate the 8h-TWA. Smoking, non-occupational asthma and allergy were assessed. Exposure to phosgene

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Bodner et al., 2001)	Longitudinal, data taken from routine medical surveillance examinations offered every 1 to 2 years Cross-sectional analyses (symptoms before entry and at last examination) Data from 1971-1997, mean follow-up ca. 8 years Dow Chemical, Texas, USA 305 TDI-exposed workers 581controls (hydrocarbons department)	TDI Manufacture	Mean observation period of TDI workers 7.8 years (SD 6.2) 449 8 h TWA TDI samples in 20 job categories; mean TDI exposure values per category calculated for start-up period (1971-1979) and full production period (1980-1997); individual work histories were matched to the 20 job categories to produce average exposure estimates and cumulative exposure estimates for each work segment for each worker Mean TDI concentration per individual: 2.3 ppb (SD 1.0), max. 5.2 ppb Average cumulative TDI exposure: 96.9 ppb-months (SD 110.6), max. 639 ppb-months Quartiles of the cumulative TDI estimates: 1-29 ppb-months, 30-70 ppb-months, 71-133 ppb-months, > 133 ppb-months Exposure categories with cut-points at 1 ppb for 1, 5, and 10 years, expressed in ppb-months (distribution for all observations): 1-12 (8.3%), 13-60 (36.6 %), 61-120 (27.1 %), > 120 (27.0 %)	**Clinical symptoms** (questionnaire):One of the symptoms significantly more prevalent in controls than in exposed subjects at baseline (shortness of breath). Prevalence for all symptoms increased in both groups over time. Prevalence of symptoms not higher in TDI exposed subjects compared to controls at final examination. No effect of TDI on clinical symptoms reported during the study period found in regression models using four cumulative exposure categories or using a continuous cumulative variable or using quartiles of exposure. **Lung function** (spirometry):Average annual decline in FEV_1 was 30 mL. No association of TDI and decline in lung function found with mixed regression models using different exposure terms and subgroups.	Longest follow-up time (together with Ott et al. 2000) for TDI workers until then. Retrospective (change of formats of health surveys) Not enough exposure samples to derive annual TDI concentration estimates for each year for each job category Regression analyses for symptoms were adjusted for observation period and pack-years. Covariates considered for the mixed models for longitudinal lung function change were initial FEV_1, initial FVC, age, observation period, height, race, sex, race, entry period, pack-years, asthma, shortness of breath No exposure to MDI (as in some foam-manufacturing operations)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Clark et al., 2003)	17-year longitudinal 1981-1998 UK Follow-up of Clark et al. 1998 7/12 factories remained n = 251 (217 were in the previous study)	TDI Manufacture of PU foam	Personal measurements: n = 1004 valid 1.3 % in excess of 46.4 ppbh (5.8 ppb, 0.02 mg NCO/m^3) Respiratory protection taken into account by subtracting 50 % of calculated exposure values Average daily dose for each exposed job at each factory calculated from the current and previous measurements Mean exposure for the period: Exposed group (n = 175): 8.4 ppbh Handling group (n = 26): 4.8 ppbh Low exposure group (n = 11): 2.3 ppbh	Longitudinal decline in **lung function** (same spirometer as in previous study; earliest measurement during 1981-1986 + further measurement in 1997/1998 used): Significantly higher loss in FEV_1 and FVC in handling group vs. low exposure group. Annual decline of FEV_1 and FVC not associated to TDI exposure. **Respiratory symptoms** (questionnaire): Differences in prevalence of respiratory symptoms between initial and final survey (reduction in some, increase in other symptoms).	Study was not designed to identify cases of sensitisation Persons showing evidence of TDI sensitisation would be removed and would no longer be available for study High attrition rate Respiratory illness was the reason for leaving in 2.3 % of cases 70 subjects out of 251 (28 %) changed groups during the 17-year period Number of present smokers fell from 129 (51 %) to 100 (40 %) between the two studies Only two data points used for lung function decline
(Dragos et al., 2009)	Prospective inception cohort study, 18 months n = 385 apprentice car-painters recruited between 1999 and 2002, complete data for n = 298	HDI monomers (and oligomers)	Personal breathing zone samples (n = 51) during regular and specific activities Area sampling (n = 41) in spray cabins and workplace background Duration for effective exposure to HDI max. 7 months, median 3 months	Health assessment included: - Respiratory symptoms (questionnaire) - Lung function (spirometry) - Metacholine challenge - Skin prick tests (only first visit) - HDI-specific IgE, IgG and IgG4	Subjects lost to follow-up 21.5 % Short observation period Pre-exposure possible No individual exposure estimates

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Dragos et al., 2009), ctd.	First visit upon entry and second visit at the end of the training programme Montreal area, Canada		Median (maximum) concentration in µg/m^3, personal samples: Monomer: Spraying 0.001 (0.006) Mixing 0.0003 (0.0003) Brush cleaning < LOD (Oligomer: Spraying 0.283 (0.916) Mixing 0.4365 (0.6890) Brush cleaning 0.079 (0.079)) Concentrations from area sampling were lower than from personal sampling	Aims: - describe changes in specific antibodies to HDI - describe incidence of work-related symptoms - examine association between work-related symptoms and changes in specific antibody levels, and other potential risk factors Increases in specific IgE and IgG levels > 97th and 95th percentile were significantly associated with duration of exposure (nine subjects increased their IgG levels /IgE levels above the cut-off of the 97th percentile). Increases in specific IgG and IgG4 showed a protective effect on the incidence of work-related lower and upper respiratory symptoms, respectively. 13 subjects (4.4 %) developed work-related respiratory symptoms, 19 (6.4 %) developed work-related symptoms of rhinoconjunctivitis. No association between change in IgE levels and incidence of symptoms.	Masks worn when spraying, but not always those recommended and often removed inappropriately for inspecting the work. In regression analysis (dependent variable: IgE or IgG) only duration of exposure was used, but no concentration. At the exposure level in this study and after a few months, a small proportion shows increases in HDI-specific IgG and IgE
(Cassidy et al., 2010)	Matched retrospective cohort study Expands on Hathaway et al. 1999 (includes an additional plant)	HDI Two plants manufacturing or producing monomer (and/or polyisocyan-ates)	Industrial hygiene personal samples If record indicated that respiratory protection was used, sampling record was not considered	**Asthma** (annual medical surveillance history forms; suspect cases were inspected further by a company physician): No new asthma cases were reported.	No quantitative exposure estimations on the individual level Small number of exposure samples to reflect whole study period

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Cassidy et al., 2010), ctd.	Observation period: Plant 1 1988-2007 Plant 2 1987-2006 Southern US 57 potentially exposed in plant 1 and 43 in plant 2 (mainly exposed to HDI monomer) Controls: Plant workers without documented history of exposure to diisocyanates 1:1 matching by age, gender, race, smoking status, date of birth, date of hire		Mean (range): Plant 1, 237 samples 0.79 ppb (Non detectable – 31 ppb) Plant 2, 29 samples 0.3 ppb (Non detectable – 2 ppb) Most of the study group reported some instances of dermal exposure	Changes in **lung function** over time (annual spirometry), examined by a random coefficient regression model: Decline in lung function (FEV_1, FVC) over time in the exposed group was significantly greater than in the control group.	Smoking was assessed as binary variable. Controls may have been heavier smokers (significant difference in lung function decline between smoking controls and smoking exposed) Potential co-exposures reported: Exposed group: Other aliphatic diisocyanates, HDI polyisocyanates Control group from plant 1: dinitrotoluene, hydrazine, methylene chloride, maleic anhydride, toluene diamine, ethylene oxide Control group from plant 2: cerium, neodymium oxides, nitric acid, ammonia, kerosene, tributyl phosphate (depending on work area) No employee had to be medically removed because of HDI exposure Individuals with asthma were excluded from work with potential exposure (only in plant 1) and there may have been self-deselection.
(Gui et al., 2014)	Inception cohort study	TDI-based state-of-the-art PU foam	Continuous fixed-point air sampling in foaming hall and cutting areas.	Over the first year of employment, 7 workers (14 %) had findings that could indicate TDI-related health effects	Actual exposure of individuals is not known: TDI air levels may have been higher near the

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
	Evaluation of 49 newly hired workers pre-employment, after six and after twelve months Grouping of workers in exposure risk groups, based on potential risk of TDI exposure: low n = 8, medium n = 28, high n = 13.	production in Eastern Europe	90 % of the samples < LOD (0.1 ppb) Maximum recorded 10.0 ppb (foaming hall), 5.4 ppb (cutting area) No air sampling period exceeded an 8 h TWA of 5 ppb Peak exposures recorded were below 20 ppb. Personal sampling performed on seven workers. All showed TDI levels < LOD. Dermal exposure occurred (uncured or just cured foam, contaminated surfaces).	(Either new asthma symptoms, TDI-specific IgG, new airflow obstruction or a decline in $FEV_1 \geq 15$ %). Twelve workers (25 %) were lost to follow-up. Among these workers, current asthma symptoms were reported (at baseline or 6 months) in a significant higher percentage compared to those who completed the 12-month follow-up. No significant associations were found between the exposure risk group and health outcomes. Self-reported glove use differed significantly between the exposure risk groups (25 % of the workers in the low, 32 % in the medium, 100 % in high exposure risk group). Although this production facility is reported to be state-of-the-art with exposure below the OEL, the study suggests possible TDI-related health-effects.	source. Dermal exposure occurred. Glove use differed between exposure risk groups. No unexposed control group No exposure quantification per exposed group Workers with spirometry data at baseline n = 23, with spirometry data at all three time points n = 16. Baseline spirometry conducted at another facility.

1.1.2.3 Case-control studies

The available case-control studies are summarised in Table 5.

Table 5: Case-control studies on respiratory sensitisation related to HDI, MDI, or TDI

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Tarlo et al., 1997)	Comparison of the level of isocyanate Concentration in 20 "case companies" (with compensated isocyanate asthma claims) with 203 "non-case companies"	HDI, MDI, TDI (or more than one)	Exposure data taken from a database of the Ontario Ministry of Labour (MOL): Air samples collected during the same 4-yr period during which the OA claims arose. Exposure determined on the basis of the highest level identified. Two categories: Always < 0.005 ppm Ever ≥ 0.005 ppm	56 accepted claims for OA (OA cases with identified isocyanate exposure during the 4-year period from mid-1984 to mid-1988 in the Ontario Workers' Compensation Board) Combined across isocyanate types: Companies with claims in the high exposure category: 10/20 (50 %) Companies without claims in the high exposure category: 50/203 (25 %) OR = 3.1 (95 % CI: 1.1–8.5, p = 0.03). MDI: OR = 1.7 (95 % CI: 0.4–7.6) TDI: OR = 2.7 (95 % CI: 0.7–10.6) Estimated incidence of OA in a 4-yr study period: High exposure companies with claims: 2.7 % Low exposure companies with claims: 2.2 % Overall incidence in the total 223 companies surveyed: 0.9 % (56 out of 6308 workers).	Many high exposure companies without claims. Other factors may be important in isocyanate sensitisation, or there may have been quantitative or qualitative differences in exposure that were not assessed. Selection bias possible (some of the air sampling conducted in investigation of submitted claims for OA) Companies with claims had more employees than those without claims (higher probability of at least one employee becoming sensitized in a greater group of employees; larger companies may be more likely to implement a surveillance program).
(Meredith et al., 2000)	Company A: 27 OA cases were matched to 51 references (sex, work area)	Company A: 24 cases attributed to TDI (manufac-ture of moulded and block flexible PU foam, flame bonding and surface coating of fabrics);	Company A: Personal exposure measurements by job category (1979-1986) made for a separate study + data collected after 1986 by occupational hygiene consultants were used to estimate 8h-TWA and	**Asthma** Data from the two sites were analysed separately. Company A: Conditional logistic regression: 8 h TWA as a binary variable (cut off: median concentration in control group) or continuous variable 0.1 ppb increments)	Uncertainties in exposure assessment Regression analyses adjusted for smoking and different atopic diseases

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Meredith et al., 2000), ctd.	Company B: 7 cases; all non-cases (n = 12) served as controls, because matching was not possible (moving between work areas, few workers)	3 cases attributed to MDI (batch moulding of rigid PU components at 200 °C) Company B: Cases attributed to MDI from a chemical plant in which MDI and poly-merric MDI mixtures were pro-cessed and poured into drums. Some processes involved heating the mixtures.	peak exposure for each subject based on job title and date. Company B: Personal monitoring results from 1988 available (Marcali method to the middle of 1990, HPLC thereafter) For each subject, the proportion of measurements ≥ LOD of the Marcali method (2 ppb) and > 5 ppb were calculated. Measurements < 2 ppb were treated as being 0. 90 % of the 269 TWA samples were < 2 ppb	Peak exposures: 1 – 50 ppb In 31 subjects peak exposure > 20 pbb No difference between cases and controls. Mean 8-h TWA: cases: 1.5 ppb; controls: 1.2 ppb OR for exposure > median of the control group: 3.2 (95 % CI 0.96 – 10.6; p = 0.06) Adjusted OR (for 0.1 ppb increase in 8h-TWA): 1.07 (95 % CI 0.99 – 1.16) Adjusted OR higher for smoking (2.4) as well as history of either hay fever, eczema or asthma (3.4), but also n.s. Company B: Association between reported chemical accidents and asthma.169/185 TWA samples for controls and 74/84 for cases were < 2ppb. Mean and median exposures were < LOD for cases and controls. Median of the highest concentration recorded for each subject was 3 ppb for both groups. Proportion of measurements ≥ 2 ppb was 0.09 (controls) and 0.18 (cases). Proportion of measurements > 5 ppb was 0.004 (controls) and 0.09 (cases). 3/7 cases and 1/11 controls had at least one 8h-TWA exposure measurement > 5 ppb (OR 7.5; p= 0.09)	Amines are used as catalysts in the manufacture of PU foams and they have been reported to cause respiratory symptoms

1.1.2.4 Cross-sectional studies

The available cross-sectional studies are summarised in Table 6 and Table 7.

Table 6: Cross-sectional studies with quantitative exposure-response estimates on respiratory sensitisation related to HDI, MDI, and/or TDI

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Pronk et al., 2007)	n = 581 (241 spray painters, 50 unexposed office workers, and 290 others) Workplace survey in several companies between 2003 and 2006	HDI monomer and trimers in spray-painting (car body repair shops, furniture paint shops, industrial paint shops specialising in ships and harbour equipment or airplanes)	Personal exposure estimates were obtained combining personal task-based inhalation measurements for 23 different isocyanate compounds and time activity information Exposure of 241 spray painters, [µg NCO * m-3 * h * mo-1], median (min-max): Total isocyanate 3,682 (4-66464) HDI 27 (0.2-1427) (Biuret 269 (0.2-13568) Isocyanurate 2250 (6-87623))	Prevalence ratios (PR) and 95 % CI for an interquartile range increase in exposure were calculated based on log-transformed exposure data. **Respiratory symptoms** (grouped into “asthma-like symptoms” and “COPD-like symptoms”), work-related symptoms (questionnaire): Respiratory symptoms were more prevalent in exposed workers than in office workers. Significant positive log-linear exposure-response associations were found for: Asthma-like symptoms PR (95 % CI) = 1.2 (1.0-1.5), COPD-like symptoms 1.3 (1.0-1.7), Work-related chest tightness 2.0 (1.0-3.9) and Work-related conjunctivitis 1.5 (1.0-2.1), but not for Work-related rhinitis 1.3 (0.9-1.7) Different HDI-specific (for monomer and oligomers) IgE and IgG antibodies:	For subsample with BHR see (Pronk et al., 2009) Prevalence ratios were adjusted for age, sex, current smoking and atopy (or some of those) Possible effect modification by atopy was explored

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Pronk et al., 2007), ctd.				Prevalence of specific IgE antibodies was low (up to 4.2 % in spray painters). Prevalence of specific IgG was higher (2-50.4 %). One of five specific IgE antibodies and four of five specific IgG antibodies were positively associated with exposure. **Bronchial hyperresponsiveness (BHR)** assessed by methacholine challenge in a subset of 229 workers. Individuals with asthma-like symptoms were more likely to have BHR: PR (95 % CI) = 2.2 (1.5-3.2). For COPD-like symptoms, the association with BHR was less strong and n. s.	
(Pronk et al., 2009)	Subset of study by Pronk et al. 2007 229 workers from 38 companies (91 spray-painters, 20 unexposed office workers, 118 others)	HDI monomer (and trimers) in spray-painting	Personal exposure estimates were obtained combining personal task-based inhalation measurements for 23 different isocyanate compounds and time activity information Exposure of 91 spray-painters, [µg NCO/m^3 x h/mo], median (min-max): Total isocyanate 4530 (15.4-66464) HDI 36.2 (1.3-472)	Prevalence ratios (PR) and 95 % CI for an interquartile range increase in exposure were calculated based on log-transformed exposure data. **Lung function**: Highly exposed workers had lower FEV1, FEV1/FVC and flow-volume parameters. Percentage of workers who met the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria for COPD (FEV1/FVC <70 %): Office workers 5, other workers 4, spray-painters 15. COPD clearly associated with exposure. PR (95 % CI): 2.7 (1.1-6.8) **Bronchial hyperresponsiveness** (BHR) (defined as a provocative cumulative dose of methacholine of ≤ 2.5 mg (~10 µM) required to cause a 20 % fall FEV1): Percentage of workers with hyperresponsiveness (BHR20): office workers 0, other workers 14.7, spray-painters 20.	Associations were adjusted for age, sex, current smoking and atopy Associations for lung function parameters: additionally adjusted for height and race Strengths: Quantitative inhalation exposure assessment based on > 500 measurements and detailed task activity information; Several objective respiratory effect measures investigated in one population Limitations: Use of personal protective equipment, previous exposures and dermal exposure was not taken into account; Complex exposure environment; Healthy worker effect possible

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Pronk et al., 2009), ctd.				Hyperresponsiveness was found in 33 subjects and it was clearly associated with exposure expressed as total NCO. PR (95 % CI): 2.0 (1.1-3.8) (adjusted for smoking, age, sex and atopy) BHR combined with asthma-like symptoms was present in 19 subjects and the adjusted PR was 2.7 (1.0-6.8). **Symptoms** (see (Pronk et al., 2007)): Asthma-like symptoms, COPD-like symptoms, work-related chest tightness were more prevalent among workers with higher exposure (n. s.). Workers with asthma-like symptoms had sign. more BHR, sign. lower baseline FEV1, FEV1/FVC and maximal mid-expiratory flow. No sign. association between exposure and exhaled nitric oxide (eNO) IgE and IgE (see (Pronk et al., 2007)): The prevalence of specific IgE antibodies was low (< ~4.4 %). The prevalence of specific IgG was higher (up to 47 % in spray painters). Specific IgG sensitisation was more common in highly exposed workers. Workers with specific IgE/IgG were more often hyperresponsive (overall; statistically significant only for one IgG). *"The current study provides evidence that exposure to isocyanate oligomers is related to asthma with bronchial hyperresponsiveness as a hallmark, but also shows independent chronic obstructive respiratory effects resulting from isocyanate exposure."*	

Table 7: Further studies - cross-sectional studies

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Bruckner et al., 1968)	Cross-sectional n = 26 with multiple exposures to diisocyanates n = 18 had never worked with or around isocyanates	TDI, polymeric isocyanates including MDI, xylylene diisocyanate Research, development and production of isocyanates and other components of urethane plastics	Exposed workers had accumulated exposure from 3 months to 11 years Air samples taken by industrial hygienist, modified Marcali method. Between 3 and 79 samples per year for single years between 1957 and 1967. Median concentration per year: 0-77 ppb	**Symptoms** (interview, physical examination) Immunologic reactivity to isocyanate antigen conjugates (several tests) Four groups: - Exposed minimal response (minimal symptoms of mucous membrane irritation) n = 5 - Exposed overdose response (moderate to marked signs and symptoms of chemical irritation of the respiratory tract) n = 16 - Exposed sensitised (signs and symptoms of sensitisation) n = 5: With increasing number of exposure, the time to reaction became shorter and finally bronchospastic symptoms developed within seconds after exposure to minute amounts of isocyanates. All had irritative symptoms before developing symptoms indicative for sensitisation. All had exposures > 20 ppb. - Non-exposed n = 18 6 cases of irritant dermatitis Workers exposed to low levels (not given) of isocyanates developed eye, mouth and throat symptoms. According to the authors concentrations between 20-100 ppb “may predispose some workers to sensitivity to isocyanate compounds”	Groups built based on exposure and type of response
(Wegman et al., 1974)	Cross-sectional 1972 Before and after shift on a Monday after three days away from work n = 111 (78 males)	TDI Manufacture of PU for matresses and auto seat cushions	Area sampling on the day of lung function testing and on three subsequent days (Marcali method, (Marcali, 1957)) All job areas were sampled and assigned exposure values and each worker was categorised according to his or her exposure to a measured mean concentration of TDI.	**Lung function** (spirometry: FEV_1, FVC; in the morning before work and in the afternoon after eight hours work; only FEV_1 reported): All exposure groups showed significant loss in lung function (FEV_1) during the working day. Dose-response relationship suggested (mean change in FEV_1 0.078 L in group A and 0.180 L in group D). Confirmed by regression analyses. And con-firmed by calculation of ratios of those showing no change or increase over those showing decrease per exposure group (ratio increases with exposure group).	Followed up: (Wegman et al., 1982; Wegman et al., 1977) Age, height, years smoked, cigarettes smoked, duration of exposure was considered for stepwise regression analysis

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wegman et al., 1974), ctd.			Originally exposure categories were combined to four groups (ppm): A 0.002 - 0.003, B 0.004, C 0.005, D 0.006 – 0.013	Greater fall in FEV_1 in workers with symptoms compared to workers without symptoms, n. s. No trend of FEV_1 across subgroups of age, years of smoking or years of employment.	
(Pham et al., 1978)	Cross-sectional Two factories producing mainly plastic foam automobile accessories 318 workers (214 men) who had been employed for at least a year	MDI PU foam moulding	Workers used MDI and some TDI for 1 to 10 years. Plant A: MDI consistently < 20 ppb Plant B: MDI peaks up to 87 ppb at foam injection workplaces Group I: Not exposed to any occupational hazard n = 83 (62 men) Group II: Indirect exposure risk due to foam plastics manufacture n = 117 (61 men) Group III: Definite, direct exposure risk due to foam plastics manufacture n = 118 (91 men)	**Lung function** (single breath carbon monoxide transfer factor test, spirometry): Lower values of VC and diffusion constant in the exposed groups and associated with length of exposure. Possibility of fibrosis in workers with long exposure suggested. Results for men not confirmed by results for women. **Respiratory symptoms** (questionnaire): Higher frequency of bronchitis in exposed groups compared to unexposed group (men and women).	Followed up by (Pham et al., 1988) Exposure on factory level Men and women analysed separately Exposure to stripping agents, solvents, polyvinyl vapour in exposed groups Exposure to TDI No statistically significant differences between the groups concerning age, height, weight, smoking. More men smoke than women and they are heavier smokers.
(Holness et al., 1984)	Cross-sectional, shift, intraday, intraweek 1982 Toronto area Four companies	TDI Use in foaming operations	Mean length of exposure to isocyanates of 6.5 years Monitoring of TDI and respirable dust during same shift as lung function analysis (area samples; personal samples for 86 workers)	**Lung function** (spirometer, beginning and end of work shifts on Monday, Wednesday, Friday, sitting position using noseclips): Values of all lung function parameters (Monday morning) lower in the exposed than in the control group (not significant, adjusted for smoking). Significantly larger declines in lung function over the shift in exposed workers.	Respirable dust, mean for all exposed: 0.30 mg/m^3 Significantly lower frequency of family history of asthma, hay fever, bronchitis in exposed group (may be due to screening prior to employment or workers with positive family history may have developed symptoms and left).

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Holness et al., 1984), ctd.	95 isocyanate-exposed workers (70 % males, 26 foam-line, 11 injection, 28 finishing, 21 miscellaneous) 37 control workers (62 % males; 16 plant, 21 Ministry of Labour) (29 were excluded)		Mean exposure concentration for five groups of workers: Area: 0.1 – 1.8 ppb Personal: 0.6 – 2.1 ppb Mean for all exposed: Area: 0.6 ppb Personal: 1.2 ppb Some analyses with three exposure categories: control, ≤1ppb, >1ppb One personal sample > 20 ppb Less than 3 % of the personal or area values > 5 ppb	Decline in FVC and FEV_1 over the shift increased over the three exposure categories, but was statistically significant only between controls and exposed groups. No significant relationships observed in regression analysis with continuous exposure. **Respiratory and further symptoms**: Slightly higher frequency of respiratory symptoms in exposed group, n. s..	
(Alexandersson et al., 1985)	Cross-sectional n = 67 (57 males) n = 56 controls (11 with lung function tests)	TDI, MDI Seven PU foam manufacturing factories (two foam PU blocks, five cast PU in moulds)	Personal sampling on same day as lung function tests Day mean exposure to TDI in foaming of PU blocks: for the whole group: 0.008 mg/m^3 (0.001 ppm) Highest exposure in the group working by foaming machine: 0.023 mg/m^3 (0.008-0.060) Day mean exposure to MDI ≤ 0.001 mg/m^3 during casting in moulds. Highest measurement: TDI 0.275 mg/m^3 MDI 0.139 mg/m^3	**Lung function** (spirometry: FEV_1, FVC, FEV %, MMF; nitrogen washout: Phase III, Closing volume; in the morning prior to work; exposed workers were studied again in the afternoon after work): Lung function of non-exposed group similar to reference values. Lung function of exposed group significantly impaired as compared to reference values, but significant in subgroup of smokers only. No significant changes during work shift. **Symptoms** (standardised questionnaire): Frequency of symptoms significantly higher in exposed non-smokers than in non-exposed non-smokers (nose, throat, dyspnea). No significant difference in symptoms frequency between exposed and non –exposed smokers.	To calculate day exposure figures < detection limit (0.001 mg/m^3) were set to zero. Selection bias (underestimation of acute adverse effects of TDI as sensible individuals may tend to terminate their employment)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Venables et al., 1985)	Cross-sectional (Outbreak of asthma was investigated) 1979 n = 221	TDI Steel coating plant; continuous process, coat was cured by passage through an oven	TDI: 14 ppb at oven entry during normal processing, up to 26 ppb during 5 minute stoppage TWA 1979: 20 ppb	21 workers (9.5 %) with OA symptoms (questionnaire) in 7 years (onset of symptoms after 1971) Symptomatic groups had significantly lower FEV_1 than asymptomatic group. TDI was found to be the cause of the asthma outbreak. It was liberated by a coating modified by a supplier in 1971.	No individual exposure levels Affected individuals may have left the plant
(Alexandersson et al., 1987)	Cross-sectional and over workweek 15 garages in Stockholm area n = 41 car painters n = 48 car platers (exposed to solvents, grinding dust, welding fumes like car painters, not to isocyanates n = 70 car mechanics Car painters and platers were matched against a control by sex (only males), age, height, and smoking	HDI Monomer (and biuret trimer) Car painters working with polyurethane paints	Exposure questionnaire Exposure monitoring 278 samples of HDI (and HDI-BT) Exposure has been individually related to time, use of respiratory protections, working operation, ventilation. Individual exposure determined by industrial hygienist HDI: 1.0 µg/m^3 (HDI-BT for car painting: mean (range): 115 µg/m^3 (10-385) High short-term peaks up to 13500 µg/m^3 HDI-BT)	Exposed workers were examined on Monday morning before work and on Friday afternoon Change in **lung function** within the week (spirometry: FEV_1, FVC, maximum mean expiratory flow MMF; Nitrogen washout: Phase III, Closing volume): Car painters did not differ from controls in any of the spirometric variables (before the workweek). Closing volume percent was significantly higher in exposed than in control workers. No significant difference in lung function in car painters before and after a workweek. **Symptoms** (interview by a nurse, standardised questionnaire): Eye, nose, throat irritation more frequent in car painters and platers than in controls, significant for platers only.	Uncertainties in exposure assessment Selection bias (some car painters had been relocated or their employment terminated)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wang et al., 1988)	Cross-sectional 1985 Taiwan n = 34, mostly females (38/45 workers had complete data, 4 were excluded because of smoking history) Follow-up (five months after recommendations for improvement of worker protection by the study team)	TDI Velcro-like tape manu-facture	Average length of employment 9.2 months Air samples, mean: Weaving (n = 3) 12 ppb Packaging/storage (n = 3) 21 ppb Tape processing (n = 15) 47 ppb Highest concentration measured: 236 ppb 5 months after improvement: 7 of 9 air samples < 7 ppb at the processing area	**Lung function** (spirometry in the morning, during a usual working day, after 10 days holiday,5 months after improvement of the workplace): Lung function of n = 21 workers after 10 days holiday: Greatest changes in pre- and post-exposure FEV1 and FVC for workers in the processing areas **Asthma** or asthmatic bronchitis (defined by development of cough for more than 1 month and shortness of breath or wheezing for 1 month after working in the factory):14 workers met the case definition of asthma or asthmatic bronchitis. Overall prevalence of asthma = 14/34 = 41.2 % Significant trend in asthma frequency across the three exposure areas (0 cases in weaving, 37.5 % in packaging/storage, 84.6 % in tape processing). Follow up (5 months): No asthmatic symptoms. Lung function significantly improved (FEV_1 and FVC) for 10 workers still employed.	No unexposed control group Difficult to distinguish between irritant and allergic reactions Reversibility may be due to irritant effect and due to short exposure duration. High turnover rate
(Olsen et al., 1989)	Cross-sectional Dow, Texas, USA n = 57 manufacturing workers (85 % participated) n = 89 unexposed workers (89 % participated)	TDI Manufacture operations	Average TDI plant experience 4.1 years (< 1 – 9 years) Routine industrial hygiene measurements: TWA < 5 ppb, short-term exposure level 20 ppb for routine plant processes Use of self-contained breathing apparatus for breaking into lines for employees. Potential exposure was ranked by an industrial hygienist: None, low, moderate, high	**Lung function** (spirometer, after at least two days away from work, standing or sitting, without the use of nose clips): TDI exposure (classified as current, highest, cumulative, cumulative highest-to-date) not associated with decline in FEV_1 **Respiratory symptoms** (questionnaire): Prevalence of upper respiratory symptoms 68 % in nonexposed group, 34 % in exposed group Prevalence of lower symptoms 33 % in nonexposed group, 17 % in exposed group	No individual exposure levels Age, height, smoking considered in regression analysis Exposure misclassification possible, because rankings were applied to jobs regardless of calendar time

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Huang et al., 1991)	Cross-sectional 1988-1989 Asia 48 workers (25 males) in three factories: Factory A n = 15 Factory B n = 29 Factory C n = 13 18 controls (9 males)	TDI Furniture manufacture factories; painters exposed to TDI aerosol while brushing PU varnish to the surfaces of wood furniture	Area sampling at five spots Day mean exposure calculated from four measurements taken one, three, five, seven hours after the start of the work shift Marcali method Mean (range): Factory A: 0.79 mg/m^3 (0.49-1.18) Factory B: 0.31 mg/m^3 (0.22-0.89) Factory C: 0.11 mg/m^3 (0.07-0.24) Aerosol Dermal exposure likely (at least in factories A and B)	**Lung function** parameters (spirometry): Impairment of some lung function parameters significant in workers of factories A and B compared to the control group. **Symptoms** of the respiratory tract, skin, eyes (structured questionnaire administrated by occupational physicians): Prevalence of symptoms was significantly higher in factory A as well as in factory B compared to the control group. No significant difference was detected between workers in factory C compared to the control group. Symptoms of the eyes, nose, throat in all workers in factory A, 60 % in factory B. No symptoms of the eyes in factory C and in the control group, 11 to 15 % reported symptoms of the nose or throat. **Asthma-like symptoms** (dyspnea and wheezing during work):4 workers (26.7 %) in factory A 3 workers in factory B (15 %) no subject in factory C and of the control group. **Patch test** (0.1 % TDI): Positive patch test in 5 and 2 painters in factories A and B (including three and two workers with contact dermatitis, respectively) and no subject in factory C or the control group. **Mast cell degranulation test**: Significantly higher mast cell degranulation percentage (MCDP) in painters from factories A and B than for the controls (specific to TDI-OA conjugates). No significantly higher MCDP in painters in factory C compared to the control group.	Cited in (Diller, 2002) Exposure measured only on one day and not on an individual level High exposure levels make it difficult to differentiate between irritant and allergic reactions. No information on potential differences in PSA between the factories. Medical history, smoking habits, duration of exposure, weight, height, age were assessed. No subject had a history of respiratory or skin diseases.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Parker et al., 1991)	Cross-sectional Minnesota, USA 39 randomly selected autobody repair shops (out of 139 contacted shops 59 were eligible) 162 workers (160 males)	MDI, TDI Autobody repair	Mean number of years in autobody industry 11.4 ± 9.7 Isocyanate samples from 32 shops 8 h TWA total isocyanates: not detected to 60 ppb, mean 5 ppb Four percent of workers who spray-painted at least one hour/week never used a respirator, 33 % sometimes, 63 % always.	**Lung function** (spirometry at the start and the end of the work day): Abnormal lung function (< 5th percentile) in 8 % (FEV1, FVC) and 23 % (FEV1/FVC) of never smokers. No significant change in lung function between morning and afternoon shifts. Working-years in the autobody industry, nonfunctioning spray booth, smoking were associated with a decrement in FEV1/ FVC (regression analysis). No relationship between shop isocyanate concentration and lung function. **Respiratory symptoms** (self-administered questionnaire): Significant increase of wheezing across categories of respirator use (always, sometimes, never) while spray painting and for coughing and wheezing while sandblasting for non-smokers. No trends for respiratory symptoms and respirator use while sanding.	No individual exposure levels Exposure to dust, solvents
(Lee and Phoon, 1992)	Cross-sectional 26 exposed workers (“mixers”), 26 controls (workshop maintenance and field staff from government departments), matched by age, race, smoking state	TDI PU foam manufacture	24 personal breathing zone samples: Mean: 0.16 ppm Range: 0.01 – 0.50 ppm	**Lung function**: Mean diurnal variation in PEFR (in one week period): Significantly higher diurnal variation in PEFR in mixers than in controls. FEV_1/FVC significantly lower in exposed (83.0 %) than in controls (89.3 %)	Cited in (Diller, 2002) High exposure level Survivor population

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Lee and Phoon, 1992), ctd.				Mixers with ten or more years of exposure showed evidence of chronic airways obstruction. **Respiratory symptoms** (questionnaire): About 50 % of mixers had eye irritation or cough during work (significant higher prevalence than in controls). No overt cases of OA	
(Omae et al., 1992)	Cross-sectional (4-year follow up see (Omae et al., 1992)) 1981 Japan 90 workers (male), 44 reference workers in the same factories	TDI PU foam manufacture	Working in PU foam factories for 0.5-25 years, mean 13.3 129 personal samples: Arithmetic mean: 3.2 ppb, geometric mean: 1.0 ppb , 90th percentile: 8.4 ppb, maximum: 26 ppb Short-term exposure peaks > 20 ppb in 16/129 samples	**Lung function**, change over working day (three methods: forced expiratory flow-volume test, respiratory impedance, airway resistance and specific airway conductance): No significant differences in lung function between PU foam workers and referents, except for lower PEF and % PEF in the exposed group. No change of lung function during work shift in both groups. **Symptoms** (questionnaire with interview): Significantly higher prevalence of respiratory symptoms, nasal symptoms, eye symptoms in the exposed workers.	Exposure to tertiary amines, organic tin compounds, polyols, silicon oil, dichloromethane, freons, flame-resisting agents, pigments etc. Possibly a survivor population Current smoking did not affect the results
(Bernstein et al., 1993)	Cross-sectional 1991 n = 243 (n = 175 males) 3-year old plant	MDI Urethane mould plant that had been designed to minimise exposure to MDI	Average duration of employment: 18.2 months (range: 0-32 months) Continuous monitoring of MDI area levels: < 5 ppb Occasional spills reported by workers, but not detected by monitors	Methods: Workers with at least one lower respiratory symptom (questionnaire) and workers with specific antibodies were instructed to perform serial PEFR studies for two weeks (n = 43). PEFR studies were also done in 23 control subjects (no symptoms, no antibodies). Workers with PEFR variability were evaluated by a physician (including methacholine test) for final diagnosis of OA/non-OA. Workers who were assigned final diagnosis of OA/non-OA/work-related urticaria were reevaluated in 1992 (n = 6).	No unexposed control group

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Bernstein et al., 1993), ctd.				Results: PEFR variability detected in 3/9 workers with questionnaire diagnosis of OA, in 2/4 workers with non-OA, in 2/23 control workers without symptoms. Three cases of physician-diagnosed OA (3/234, prevalence ca. 1 %) and two cases of physician-diagnosed non-OA. Two workers had specific IgE and IgG to MDI-HSA. One of those had urticaria. Cases are considered to be due to intermittent higher than normal exposures to MDI during non-routine working activities. Cases were removed from exposure. After 1 year clinical status of OA was described as "inactive".	
(Kim et al., 1997)	Cross-sectional Korea 81 workers (41 males)	TDI Spray painters Workshops manufactur-ing furniture or musical instruments or repairing motor vehicles	Area samples (n = 41) Range 0.5 – 10 ppb Mean 3.5 ± 2.3 ppb Four samples (9.8 %) > 5 ppb	Examinations: Respiratory symptoms (questionnaires and interviews), Chest auscultation, IgE, IgG, FVC, FEV_1 Diagnosis of **TDI OA** was made if there was a decrease of PEFR over 20 % of baseline and if the changing pattern was closely related to workshift. PEFR was recorded in the following cases: Subject complained of sputum, cough, and dyspnea aggravated by work, wheezing audible by auscultation, FVC or FEV_{10} < 80 % of the normal Korean reference value, positive IgE RAST for TDI PEFR was checked for 15 workers. Eight workers (9.9 %) were diagnosed with TDI-OA.	Cited in (Diller, 2002) No control group No individual exposure data

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Ulvestad et al., 1999)	Cross-sectional Norway? 19 injection workers (previous tunnel workers who were grouped into a department set up for sealing work; exposed to PU and acrylic resins; all the workers employed in this department in 1996 were included) 104 other tunnel workers, 6 different sites	MDI monomer (and prepolymer) Sealing work in tunnels	Job-years; mean (range): injection workers: 21 (1-42) tunnel workers: 13 (1-46) MDI monomer (personal sampling, 20 samples): mostly below the LOD (< 1 µg/m^3); 1.9 and 3.0 µg/m^3 at 2 occasions where isocyanate resin was spilled during injection work Pre-polymer: Four shift samples: 5.5 – 300 µg/m^3 (median 7.1); 18 short-term exposure values: 18-4300 (median 103) µg/m^3 Stationary sampling (n = 6): monomer < 4 µg/m^3, prepolymer < 4 - 31 µg/m^3	Examinations: Respiratory symptoms (questionnaire), lung function (spirometry), IgE (TDI, MDI, formaldehyde, eight common allergens), Metacholine provocation test, Clinical examination Higher prevalence of respiratory symptoms, airflow obstruction, BHR, asthma in injection workers compared to other tunnel workers. Two TDI-HSA-specific IgE positive injection workers (with work-related respiratory symptoms)	No exposure measurements available from the years the "injection department" had existed → most common exposure situations for workers during the last ten years were simulated. No individual exposure data Workers had not been informed about health hazards of the chemicals they worked with and did not report any use of airway protection. Exposure to acrylic resins Previous exposure to TDI Underestimation of exposure possible Years in the same job and smoking status were considered in the regression model
(Jang et al., 2000)	Cross-sectional Korea 64 randomly selected workers, 27 controls (23 males)	MDI (n = 20), TDI (n = 44) Petrochem-ical plant Manufacture	60 personal breathing zone samples Sampling during manufacture, sampling time 30-60 min Mean (maximum): TDI 17.4 µg/m^3 (42.9 µg/m^3) MDI µg/m^3 (6.4 µg/m^3)	Airway hyperresponsiveness (AHR) (definition: PC20 FEV_1 < 16 mg/mL of methacholine; continuous index of bronchial responsiveness: BRindex): Prevalence of AHR higher in MDI-exposed workers (4/20; 20 %) than in TDI-exposed workers (2/42; 5 %) and in controls (read from Figure: 2/27; 7 %). Significantly higher BR index in MDI-exposed workers than in controls, but not significantly higher than in TDI-exposed workers. Differences statistically significant?	No individual exposure measurements Medication, work history, atopy, smoking was assessed by questionnaire

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Kakooei et al., 2006)	Cross-sectional Iran 39 employees in an automobile manufacturing company 117 unexposed employees at other work stations	MDI Window fixation, window glue processes	Personal samples Average concentration of MDI: Window fixation 34.53 µg/m^3 Window glue workplaces 27.37 µg/m^3	**Lung function**: % FEV1/FVC, % PEF significantly smaller in the exposed group than in the control group. **Respiratory symptoms** (questionnaire): Skin, respiratory, eye, mental symptoms significantly more prevalent in the exposed group. Respiratory, eye, mental symptoms significantly more prevalent in workers exposed to higher concentrations compared to lower concentrations than the mean value of 31.22 µg/m^3. Respiratory symptoms increased with the duration of service. However, symptoms not significantly correlated to years or intensity of exposure.	Occupational health and hygiene problems due to missing application of adequate engineering controls and proper safe work practice. Study was conducted in the summer. Higher exposure levels in the winter likely, because windows are kept closed then. No significant differences between the two groups in age, height, duration of service. However, duration of service was shorter in the exposed group. No information on smoking.
(Littorin et al., 2007)	Cross-sectional Southern Sweden n = 136 exposed to TDI in eleven plants n = 118 unexposed workers from different activities	TDI or TDI-based PU MDI used in 4/5 moulding plants (low or non-detectable). IPDI used in 1 of these plants.	Median personal 8 h exposure to TDI (ppb): continuous-foaming: 0.63-4.0 flame lamination: 0.76-1.5 molding: 0.17-0.64 low heating or nonheating processes: 0.02-0.05 Individual airborne exposure: measured during one shift (n = 79 workers), estimated based on department, task, air measurements (n = 57). Biomonitoring: 2,4-TDA and 2,6-TDA Urine: LOD – 623 and 353 noml/L Plasma: LOD-254 and 509 nmol/L	**Respiratory and eye symptoms** (structured interview, physical examination): Comparison between exposed and unexposed group: Total symptoms: significant increase in symptoms of the lower airways, nose bleeding (as the only nose symptom investigated), eye symptoms for the exposed group. Work-related symptoms: strong associations with exposure, in particular for attacks of eye symptoms (OR = 10), “wheezing etc” (OR = 21) and dry cough (OR = 11). Continuous measure of exposure within the exposed cohort: Only eye symptoms significantly associated with exposure measures (air, plasma, urine; OR from 1.6 to 4.2)	Symptoms may have been caused by combined exposures. Coexposures: dusts, other diisocyanates, organic solvents, thermal degradation products of ready-made PU in flame lamination plants (mix of mono-and diisocyanates, aminoisocyanates, amines) High number of workers with airway symptoms is seen as remarkable by authors, because of the selected workforce. However, no dose-response relationship with TDI. Individual airborne exposure was measured for a part of the workers only.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Littorin et al., 2007), ctd.		5 moulding plants, 2 continous-foaming plants, 2 flame-lamination plants, 2 plants with low heating or non-heating processes	Correlations between air measurements and biomarkers in urine as well as biomarkers in plasma. Biomarkers in urine and plasma also correlated. Skin exposure certainly present	Effect of 2,4-TDI on the eyes was more pronounced compared to 2,6-TDI No clear patterns for other exposure-response relationships	Logistic regression model included age, gender, smoking. Atopy was considered. Preemployment health examinations should lead to a selected workforce in the Swedish PU industry (rather healthy concerning airway disease).
(Pourabedian et al., 2010)	Cross-sectional, shift Iran n = 43 car painters (healthy on enrolment) exclusion criteria: respiratory disorders including asthma, cigarette smoking, use of respiratory drugs	HDI Car body paint shop	Mean daily exposure: 15 minutes Mean daily HDI TWA air concentration in the breathing zone: 0.42 ± 0.1 mg/m^3 Mean weekly HDI TWA: 0.13 ± 0.059 mg/m^3	**Lung function**: Variation in PEF (peak flow meter, before and after the shift, over one week): Mean peak flow at the end of the shift on painting day was significantly lower than at the start of the shift 72 % of the workers had >10 % variation in PEF on painting days Effects of exposure remained till the day after painting Significant difference between the two days Significant correlation between HDI and percentage of decrease in peak flow as well as mean peak flow on painting day	High exposure levels No unexposed control group Questions concerning statistical analysis/ reporting of results Organic solutions

1.1.3 **Animal data for m-TMXDI**

Two of the three studies in guinea pigs available for m-TMXDI suffer from such strong limitations that they are not considered reliable by the DS. A summary in tabular form can be found in Table 9 of the main dossier.

1.1.3.1 Respiratory sensitisation study in guinea pigs

Study reference:

Union Carbide (1988): Evaluation of the potential of meta-tetramethylxylene diisocyanate (CT-291-87) to produce pulmonary hypersensitivity. Report no. 50-606, date: 1988-12-08. Union Carbide Chemicals and Plastics Company, Bushy Run Research Center. Cytec Industries, unpublished. GLP: claimed.

The text below is reproduced from the summary in the technical registration dossier, with slight editorial modifications by the DS. For a critical evaluation of the results by the DS, the reader is referred to the main part of this dossier.

Test type:

Non-guideline respiratory sensitisation study with induction and challenge performed via inhalation.

Test substance:

m-TMXDI, Analytical purity: 98.5 %, Impurities (identity and concentrations): 1, 1-dimethyl-m-isopropenyl benzyl isocyanate 3.7 %, acetyl isocyanate 0.35 %, 1,1-dimethyl-m-isopropenyl benzyl/urethane 0.2 %, m-diisopropenyl benzene 0.05 % and methoxy isocyanate 0.04 %.

Protein conjugate of test material: m-TMXDI-guinea pig serum albumin conjugate (TMXDI-GPSA) contained 79.4 % conjugate and 20.6 % buffer salts;

Test animals:

Female Hartley guinea pigs, source: Hazleton Dutchland, Inc., Denver PA, housing: one animal per cage in stainless steel wire-mesh cages (23.5 x 40 x 18 cm), diet: Pellet feed (Pro Lab Guinea Pig Diet, Agway, Inc. Delmont, PA), ad libitum, withheld during exposure, Water: Tap water (Municipal Authority of Westmoreland Country. Greensburg, PA), ad libitum, withheld during exposure, Acclimation period: 10 d.

Methods:

The animals were initially exposed to the test material by the inhalation route (induction exposure). Following a rest period of 10-14 d during which an immune response may develop, the animals were exposed to a challenge dose (inhalation). The extent and degree of reaction to the challenge exposure in the test animals was compared with that demonstrated by control animals which underwent the same treatment during induction and received the challenge exposure.

Induction

Exposure period: 3 h, Test groups: One (twelve animals), Control group: One (Eight animals, exposed to filtered air only), Frequency of applications: 3 h/d for 5 d, Duration: 5d, Concentrations: 30 µg/mL TMXDI aerosol.

Exposure chamber: Stainless steel, with glass windows for observation. Volume of the chamber: 900 L. Air flow rate: 300 L/min. Induction exposure cage dimensions: 17.5 x 24 x 18 cm wire mesh cages. Frequency of data recording: Temperature and relative humidity were recorded 6 times/exposure. Mean daily temperature: test group: 22 °C; control: 24 °C.

Challenge

Day(s) of challenge: On days 22, 23 and 26. Exposure period: Animals breathed room air for a period of 20 min, followed by 20 min exposure to an aerosol of GPSA and then exposure to m-TMXDI-GPSA aerosol for a period of 20 min. Test groups: One (Twelve animals). Control group: One (Eight animals). Concentrations: 15-20 µg/L aerosol of GPSA, followed by a 15-20 µg/L aerosol of TMXDI-GPSA after a recovery period of 30 min.

Exposure chamber: Four whole body plethysmographs attached to a 2.5 L glass primary chamber. Air flow rate: 20 L/min. Acclimation in chamber: 10-15 min. A Statham PM 15ETC differential pressure transducer was used to sense pressure changes created during inspiration and expiration of the animals; Frequency of data recording: 15 sec.

Aerosol generation of the test materials

Induction exposures: Approx. 250 mL of the test material was placed in a Laskin nebulizer operated with 100S nitrogen gas at 20 -29 psig. The aerosol entered the top of the chamber and was pulled through the Chamber by a 300 L/min diluent air stream.

Challenge exposure: Aerosols of TMXDI-GPSA and GPSA were also generated using a Laskin nebulizer. Approx. 15 mL of a 0.5 % solution (weight/volume) of TMXDI-GPSA or GPSA in distilled water was placed into a Laskin nebulizer operated with compressed air at 15 psig. A drop of pH 7.5 phosphate buffer was added to the solutions in order to ensure that the TMXDI-GPSA or GPSA was dissolved.

Analysis of chamber atmosphere

Induction exposure: Method of determination of chamber conc.: Reverse phase HPLC and gravimetric analyses. Sampling of chamber air: Through impingers containing toluene at approximately 1 L/min. No of samples: 4 for analytical determination during each 3 h exposure. Mean chamber atmosphere concentration: 31.4 ± 2.78 µg/L (by HPLC) and 30.5 ± 3.15 µg/L (by gravimetric analysis). Mean analytical to nominal concentration ratio ranged between 0.39 -0.49.

Challenge exposure: Method of determination of chamber conc.: Gravimetric analysis. Sampling of chamber air: Samples of Chamber air were drawn from a port on the top of the mixing chamber or individual plethysmograph chamber through 25 mm pre-weighed glass fiber filters at a known flow rate (1.89 L/min) for a specified time during each challenge.

Chamber concentration: Preliminary data showed that samples taken simultaneously from the mixing chamber and the individual animal plethysmograph agreed within 10 % of each other (13.1µg/L vs. 14.2 µg/L in one case; 19.0 µg/L vs. 20.0 µg/L in another case). Particle size determination: Cascade impactor (Series 210, Sierra Instruments, Inc., Carmel Valley, CA) for GPSA aerosols (respiratory challenges) and TMXDI aerosols (induction exposures). Sampling flow rate and sampling time: GPSA aerosol was 1.89 L/min and the sampling time was 60 min; TMXDI aerosol: flow rate- 8.6 L/min and the sampling time was 120 min. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined from log probability plots of the cumulative percent mass collected on each impactor stage. The line of "best fit" was calculated using a probit analysis method of Finney (1964).

Parameters observed

(a) Body weight:

Weighed on day of randomisation, each day of induction exposure and weekly during the rest of the study, and at termination. Statistics: t-test, the fiducial limit of 0.05 (two-tailed) was used as the critical level of significance for all comparisons.

(b) Respiratory rate monitoring:

All guinea pigs included in the study had respiratory rates within the range of 90-125 breaths/min. Frequency: Monitored continuously during the challenge exposure on days 22, 23 and 26.

(c) Antibody Analysis:

Blood samples were obtained from the orbital sinus of each control and TMXDI-exposed animal prior to day 1, on day 21, and at sacrifice following anesthesia with either methoxyflurane inhalation or sodium pentobarbital (i.p.). Serum was separated and frozen at approx. -20 °C and stored until the time of antibody determination. Serum was shipped to Hazleton Biotechnologies Co. on October 13, 1987, for determination of antibody titers. The samples were received by Hazleton Biotechnologies Co. on October 14, 1987. The methodology used for the enzyme linked immunosorbent assay (ELISA) to detect antibodies to TMXDI is in accordance with an internal protocol of Hazleton Biotechnologies Co. entitled.

(d) Pathological evaluation:

Day of sacrifice: On day 26, all surviving animals were sacrificed with an i.p. injection of sodium pentobarbital following challenge and examined macroscopically. Organs observed: The lungs of the animal were excised, weighed, and infused with 10 % neutral buffered formalin, followed by histopathological examination. Lungs of 2 animals that died on day 2 of the study were not necropsied. Data and Statistical Analysis: Background mean from preliminary data was 11 ± 12 % SD (Karol et al, 1980). Criteria for pulmonary hypersensitivity: Significant immediate-onset pulmonary hypersensitivity response was defined as an increase in respiratory rate greater than X + 2 SD above the animal's. baseline pre-exposure value (i.e., a 36 % increase) which is sustained for at least 3 min. A computer program (with small window) was used to smooth the data over pressure signals due to animal movement. A baseline respiratory rate value was calculated by an additional computer program which averaged the 15-sec pre-exposure interval of the inhalation challenge. Percent changes in respiratory rate during exposure to aerosols of GPSA and TMXDI-GPSA from the baseline rate were calculated every 15 seconds. Evaluation of the respiratory wave patterns also enables to distinguish between pressure changes due to animal movement and pressure changes due to the respiration of the animal.

Results and discussion:

Results

(a) Chamber exposure concentrations:

The mean induction chamber atmosphere concentrations were 31.4 ± 2.78 µg/L (by HPLC) and 30.5 ± 3.15 µg/L (by gravimetric analysis). The mean analytical to nominal concentration ratio ranged from 0.39 to 0.49 and the mean MMAD of TMXDI aerosol was 1.9 µm with a mean GSD of 2.4.

Challenge chamber concentration: Mean of individual animals was 17.48 ± 2.21 µg/L for GPSA and 15.04±2.26 µg/L (after adjusting for salt content) for m-TMXDI-GPSA. The mass median aerodynamic diameter (MMAD) for GPSA representative of exposures was 3.6 µm with a GSD of 5.75.

(b) Clinical observations and mortality:

Animals exhibited periocular, perinasal, and perioral wetness during and immediately following induction exposures. The respiration of these animals decreased and became forced during and following induction exposures. Few animals also displayed audible breathing and/or decreased motor activity during the induction exposure period.

Two animals died during the week of induction exposures (day 2) and two animals died in the time period following these exposures (days 19 and 25).

(c) Body weights:

During the period of the induction exposure, body weight loss was not significant. Following induction exposures, body weight gain was noted on days 12, 19, and 26.

(d) Respiratory rate:

During the inhalation challenge, none of the animals displayed an increase in respiratory rate greater than 36 % of their pre-exposure rate (defined evidence of hypersensitivity. In some cases the pulmonary hypersensitivity criteria were met but they were later found out to be due to animal movements).

(e) Antibody analysis:

m-TMXDI-treated guinea pigs had low, but positive titers following the induction exposures. Control guinea pigs displayed negative antibody titers. The dosing of animals could not be increased in order to increase the antibody titers, since mortality occurred in four out of the twelve exposed animals.

(f) Pathologic evaluation:

Greater incidence of alveolar histiocytosis was observed in the lungs of the TMXDI-exposed guinea pigs in comparison to control. Further atelectasis was observed in the lungs of both sacrificed and found dead guinea pigs. Microscopic examination of the two guinea pigs showed pulmonary edema (one animal), and hyaline membrane formation along with pneumonitis, congestion and hemorrhage. (Refer to Table 4-12 under 'Attached background material').

Applicant's summary and conclusion

Under the test conditions, the test material was found to be non-sensitising (Union Carbide, 1988b).

1.1.4 **Animal data for the category source substances HDI, MDI, and TDI**

Table 8 shows the complete list of animal studies initially considered for this dossier. Based on the test substance and route used for induction and further quality criteria (for details cf. main dossier), studies were selected for or excluded from further assessment.

Table 8: Overview (in chronological order) of available animal studies for diisocyanates and results of filtering for further assessment[1,2]

Species	Induction route	Induction agent	Effects observed	. Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
GP RB RA	INH	TDIuc						(Niewenhuis et al., 1965)
GP	IDE							IUCL: (Bayer, 1968)
GP	TOP							IUCL: (Bayer, 1970)
GP	INH	HMDI						IUCL: (DuPont, 1971)
GP	INH	HMDI						IUCL: (DuPont, 1974)
GP	IDE							IUCL: (Duprat et al., 1976)
GP	IDE							IUCL: (DuPont, 1977)
GP	TOP	PIPDI						IUCL: (IBR, 1977)
MO	INH	2,4-TDI						(Sangha and Alarie, 1979)
GP	IDE+ TOP	m-XDI						IUCL: (Huntingdon, 1980)
MO	TOP							(Tanaka, 1980)
GP	TOP							IUCL: (BRC, 1981)
GP	IDE TOP							(Karol et al., 1981)
MO	INH	HDI	Y	-		RF	One exposure < 1 d, no AB	(Sangha et al., 1981)
GP	IVE							(Bernstein et al., 1982)
GP	IPE SCU							(Chen and Bernstein, 1982)

[1] Studies deselected for further assessment are shaded grey, as are the fields explaining which criteria for inclusion based on test substance, route, or quality were not met (for details on the deselection strategy, cf. main dossier). If for a given induction agent and route a study contained experiments with negative test results as well as experiments demonstrating effects, only the latter have been further evaluated. Experiments with knock-out animals were not considered, since the aim of this review was to identify effects in healthy animals.

[2] For explanation of abbreviations cf. section 15 of the main dossier.

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
GP	IDE							(Karol and Magreni, 1982)
	IPE							
	TOE							
	TOP							
DO	ITR							(Patterson et al., 1982)
MO	INH	HDI-BT						(Weyel et al., 1982)
GP	IDE							IUCL: (Bayer, 1983)
GP	IDE+ TOP							IUCL: (IBR, 1983a)
GP	IDE+ TOP							IUCL: (IBR, 1983b)
GP	INH	TDI	Y	-		AB	-	(Karol, 1983)
				IDE	TDI	SS		
				INH	TDI-GPSA	RF		
GP	TOP							(Koschier et al., 1983)
GP	INA							(Tanaka et al., 1983)
GP	IDE							IUCL: (Bayer, 1984a)
GP	IDE							IUCL: (Bayer, 1984b)
GP	TOP							IUCL: (Bio-Dynamics, 1984)
GP	INH	m-TMXDI	N	INH	m-TMXDI	-	-	IUCL: (Bio-Research Laboratories, 1984a; Bio-Research Laboratories, 1984b)[3]
GP	IDE							(Chang and Karol, 1984)
GP	IDE+ TOP							(Clemmensen, 1984)
RA	INH	2,4-TDI						IUCL: (Hazleton, 1984)
GP	INH	HMDI						(Stadler and Karol, 1984)
MO	TOP +INH							
GP	IDE+ TOP							IUCL: (Bayer, 1985)
GP	TOP							(Stadler and Karol, 1985)
MO								
MO	TOP							(Tominaga et al., 1985)
MO	INH	HMDI						(Weyel and Schaffer, 1985)
		MDI	Y	-		RF	One exposure < 1 d, no AB	
MO	TOP + FCA							(Gad et al., 1986)
MO	INH	2,4-TDI						IUCL: (Hazleton, 1986)
GP	IDE							IUCL: (University of Louisville, 1987)
	INH	IPDI						
MO	TOP							(Tanaka et al., 1987)
MO	TOP							(Thorne et al., 1987)
GP	INH	TDI	Y	INH	TDI-GPSA	AB, RF	-	(Botham et al., 1988)
GP	INH	TDIuc						(Cibulas et al., 1988)
GP	IDE							(Jin and Karol, 1988)

[3] These studies – in spite of not fulfilling the general eligibility criteria – were nevertheless evaluated, since they were performed using m-TMXDI as test material, cf. section 10.6.4.1 of the main dossier.

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
RA	INH	HDI	Y	-		IF	Only IF	IUCL: (Mobay, 1988)
RA	INH	TDI	Y	-		IF		IUCL: (Union Carbide, 1988a)/ (Tyl et al., 1999)
GP	INH	m-TMXDI	Y	INH	m-TMXDI-GPSA	AB, IF, RF	-	IUCL: (Union Carbide, 1988a)
RA	INH	HDI	Y	-		IF	Only IF	IUCL: (Mobay, 1989)
MO	TOP	IPDI						(Stern et al., 1989)
RA	INH	TDI	Y	-		IF	Only IF	IUCL: (Union Carbide, 1989)/ (Tyl et al., 1999)
GP	INH	MDI	Y	-		AB	-	(Dearman and Botham, 1990)
				IPE	MDI-GPSA			
RA MO	INH	m-TMXDI	Y	-		IF	Only IF	IUCL: (Union Carbide, 1990)
RA	INH	TDI	Y	-		IF	One exposure < 1 d, no AB	(Hesbert et al., 1991)
GP	INH	HDI trimer						(Pauluhn and Eben, 1991)
	IDE							
MO	TOP							(Dearman et al., 1992a)
MO	TOP							(Dearman et al., 1992b)
GP	INA							(Kalubi et al., 1992)
GP	IDE+ TOP							IUCL: (Safepharm, 1992)
MO RA	INH	m-TMXDI	Y	-		IF, RF	One exposure < 1 d, no AB	IUCL: (Union Carbide, 1992)
GP	IDE+ TOP							IUCL: (Bayer, 1993)
GP	INH	TDIuc						(Huang et al., 1993)
GP	INH	TDI	Y	INH	TDI	IF	-	(Huang et al., 1993)
GP	INH	TDI	Y	INH	TDI	AB, RF	-	(Aoyama et al., 1994)
GP	IDE							IUCL: (Bayer, 1994)
MO	TOP							(Hilton et al., 1994)
GP	IDE							(Pauluhn, 1994)
	INH	MDI	Y	INH	MDI	RF	-	
					MDI-GPSA			
		TDI			TDI			
					TDI-GPSA			
GP	IDE							(Rattray et al., 1994)
	TOP							
	INH	MDI	Y	INH	MDI	AB, RF, SS	-	
RA	INH	PMDI						(Reuzel et al., 1994a)
RA	INH	PMDI						(Reuzel et al., 1994b)
GP	IDE	HMDI						IUCL: (Bayer, 1995a)
GP	INH	MDI	Y	INH	MDI	AB, IF, RF	-	IUCL: (Bayer, 1995b)
GP	IDE							(Blaikie et al., 1995)
MO	TOP							(Hilton et al., 1995)
RA	INH	MDI	Y	-		IF, RF	-	IUCL: (Hoymann et al., 1995)
GP	INA	2,4-TDI						(Yamada et al., 1995)
GP	TOP							(Basketter and Gerberick, 1996)
GP	IDE							IUCL: (Bayer, 1996a)
GP	IDE							IUCL: (Bayer, 1996b)
	INH	PIPDI						
MO	TOP							(Dearman et al., 1996a)
MO	TOP							(Dearman et al., 1996b)
GP	INH	TDI	Y	-		IF, RF	-	(Gagnaire et al., 1996)
MO	TOP							(Karol and Kramarik, 1996)
GP	IDE							(Mapp et al., 1996)

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
GP	INA							(Niimi et al., 1996)
GP	IDE+TOP							IUCL: (NOTOX, 1996)
MO	INA TOP							(Scheerens et al., 1996)
GP	INH	TDI	Y	-		IF	Only IF	(Ban et al., 1997)
GP	INH	TDI	Y	-		RF	-	(Gagnaire et al., 1997)
RA	INH	TDI	Y	-		IF, RF	One exposure < 1 d, no AB	(Huffman et al., 1997)
GP	IDE+TOP	m-XDI						IUCL: (Huntingdon, 1997)
GP	INH+IDE							(Pauluhn and Mohr, 1998)
	INH	TDI	Y	INH	TDI/TDI-GPSA	AB, IF, RF	-	
GP	IDE							IUCL: (Safepharm, 1998a)
GP	IDE+TOP							IUCL: (Safepharm, 1998b)
MO	TOP							(Woolhiser et al., 1998)
MO	INA							(Zheng et al., 1998)
GP	TOP							(Zissu et al., 1998)
RA	INH	PMDI						(Pauluhn et al., 1999)
MO	TOP							(Scheerens et al., 1999)
RA	INH	PMDI						(Pauluhn, 2000a)
RA	INH	HDI-IC						(Pauluhn, 2000b)
GP	IDE INH	PMDI						(Pauluhn et al., 2000)
MO	TOP+SDS	2,4-TDI						(van Och et al., 2000)
MO	TOP	2,4-TDI						(Vandebriel et al., 2000)
GP	INA							(Ebino et al., 2001)
	INH	TDI	Y	TOP	TDI	SS	-	
	ITR							
	TOP							
MO	SCU							(Matheson et al., 2001)
RA	INH	HDI-BT HDI-IC						(Pauluhn and Mohr, 2001)
RA	INA							(Zheng et al., 2001)
MO	TOP							(Haag et al., 2002)
RA	INH	PMDI						(Kilgour et al., 2002)
MO	INA							(Lee et al., 2002)
MO	SCU							(Matheson et al., 2002)
RA	INH	PMDI						(Pauluhn, 2002a)
RA	INH	HDI-IC PMDI						(Pauluhn, 2002b)
MO	TOP							IUCL: (Bayer, 2003a)
RA	INH	MDI	Y	-		RF	One exposure < 1 d, no AB	IUCL: (Bayer, 2003b)
MO	INA							(Lee et al., 2003)
GP	IDE+TOP							IUCL: (NOTOX, 2004)
MO	TOP							(Vanoirbeek et al., 2004)
RA	INH	2,4-TDI						(Kouadio et al., 2005)
MO	INH	TDI	Y	INH	TDImix	AB, IF, RF	-	(Matheson et al., 2005a; Matheson et al., 2005b)
GP	TOP							(Nabe et al., 2005)

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
RA	TOP							(Pauluhn, 2005)
RA	INH TOP	PMDI						(Pauluhn et al., 2005)
MO	TOP							(Plitnick et al., 2005)
MO	INH	TDI	Y	INH ITR	TDImix	AB, IF	-	(Ban et al., 2006)
	SCU TOP +ITR							
RA	TOP INH	PMDI						(Pauluhn and Vohr, 2006)
MO	TOP							(Selgrade et al., 2006)
MO	TOP							(Farraj et al., 2007)
MO	TOP							(Lim et al., 2007)
RA	INH	HDI-IC PHDI/ PTDI						(Ma-Hock et al., 2007)
MO	SCU							(Sun et al., 2007)
MO	TOP							(Tarkowski et al., 2007)
MO	INH	HDI	Y	-		IF, SS	-	(Arts et al., 2008)
		IPDI PIPDI						
		TDI	Y	-		IF, SS	-	
	TOP							
RA	INH	HMDI						IUCL: (Bayer, 2008a)
RA	INH	IPDI						IUCL: (Bayer, 2008b)
MO	ITR TOP							(Fukuyama et al., 2008)
RA	TOP							(Pauluhn, 2008a)
RA	TOP							(Pauluhn, 2008b)
RA	INH	IPDI trimer						IUCL: (BASF, 2009)
MO	INH	HDI	Y	-		IF, SS	-	(de Jong et al., 2009)
		IPDI						
		TDI	Y	-		IF, SS	-	
	TOP							
RA	INA							(Svensson-Elfsmark et al., 2009)
MO	TOP							(Vanoirbeek et al., 2009)
MO	TOP							(Vanoirbeek et al., 2009)
RA	INH	NDI						IUCL: (Bayer, 2010)
MO	TOP							(Fukuyama et al., 2010)
MO	TOP							IUCL: (Bayer, 2011)
MO	INH	MDI TDI	Y	-		IF, RF	Only IF and sensory irritation	(Lindberg et al., 2011)
RA	INH	PMDI						(Pauluhn and Poole, 2011)
MO	INA							(Swierczynska-Machura et al., 2012)
MO	TOP							(de Vooght et al., 2013)
MO	TOP							(Song et al., 2013)
MO	TOP							(Woolhiser et al., 2013)
MO	TOP							(Nayak et al., 2014)

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
RA	INH	TDI	Y	-		RF	Only sensory irritation	(Pauluhn, 2014)
	TOP +INH							
MO	INA							(Swierczynska-Machura et al., 2014)
MO	TOP							(Liang et al., 2015)
RA	INH	HDI	Y	-		RF	Only sensory irritation	(Pauluhn, 2015)
		HDI/PHDI						
	TOP							
MO	TOP							(Pollaris et al., 2015)
MO	TOP							(Wisnewski et al., 2015)

In the following sections, one key study for each animal species is summarised in detail[4].

1.1.4.1 Pauluhn and Mohr, 1998

Study reference:

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

Since the classification criteria for RS ask for inhalation (and not mixed intradermal and inhalation) exposure, only the experimental design and results for the two treatment groups with exclusive inhalation exposure are reported here.

Test type:

No test guideline was followed since none is available for this endpoint. Sensitisation in guinea pigs was induced by single inhalation exposure to TDI vapour with subsequent inhalation challenge with the homologous TDI–protein conjugate, immunoglobulin G_1 (IgG_1) antibody analysis, and histopathological examination of the lung. In order to distinguish specific from nonspecific respiratory response, guinea pigs were subjected to additional acetylcholine (ACh) bronchoprovocation assays one day before and one day after the challenge with TDI.

Test substance:

Toluene diisocyanate (TDI, DESMODUR T80), an 80:20 mixture of the 2,4- and 2,6-isomers, source: Bayer AG, Leverkusen, Germany, EC number 247-722-4, CAS number 26471-62-5, degree of purity > 99.9 % (identity of remaining < 0.1 % not reported), batch number not reported.

Test animals:

Guinea pigs/Dunkin-Hartley/female, weight at study initiation: 250-350 g, age at study initiation not reported, 8 animals per treatment group, 16 animals in control group.

Administration/exposure:

Route of induction and challenge: inhalation; control group: pooled from a sham-exposed group (8 animals) and a group receiving intradermal injections of corn oil (vehicle control for additional experiments performed

[4] Note: Text is a mixture of excerpts from the respective publications or IUCLID summaries and of text prepared by the DS. Direct use of original text is not specifically marked.

in this study, 8 animals); induction concentrations used in treatment groups: 136 or 220 mg TDI vapour/m^3 air; challenge 1: on day 20, unspecific challenge with acetylcholine (ACH); challenge 2: on day 21, specific challenge with 0.5 mg TDI/m^3 air for 30 min; challenge 3: on day 22, unspecific challenge with acetylcholine (ACh); challenge 4: on day 28, specific challenge with TDI-GPSA conjugate.

Results and discussion:

Following single 15 minute-inhalation nose-only exposure to TDI at two different dose levels, Dunkin-Hartley guinea pigs displayed an increased respiratory rate after specific challenge with 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 in serum samples of exposed animals. On sacrifice one day after the conjugate challenge, increased influx of granulocytes in trachea, lung and lung-associated lymph nodes and an increased number of macrophages in lung tissue were demonstrated. The results are displayed in more detail in Table 9 below (Pauluhn and Mohr, 1998).

Table 9: Results indicative of respiratory sensitisation from (Pauluhn and Mohr, 1998)

Parameter		Control	Group 1 (136 mg/m^3)	Group 2 (220 mg/m^3)
Specific TDI challenge (day 21)				
Immediate onset respiratory hypersensitivity, duration of increase of respiratory rate [5]		19 %	63 %	63 %
Immediate onset respiratory hypersensitivity, intensity of increase of respiratory rate [6]		25 %	50 %	38 %
TDI-GPSA challenge (ca. day 28)				
Immediate onset respiratory hypersensitivity, duration of increase of respiratory rate 5		6 %	25 %	38 %
Immediate onset respiratory hypersensitivity, intensity of increase of respiratory rate 6		6 %	38 %	38 %
Serum antibody production (day 28)				
Highest serum dilution demonstrating positive TDI-specific IgG_1 antibodies		NA	1:100	1:100
Histopathology				
Trachea				
Influx of granulocytes	Moderate	19 %	13 %	38 %
	Severe	0 %	0 %	50 %**
Influx of eosinophilic granulocytes	Moderate	19 %	25 %	38 %
	Severe	0 %	0 %	50 %**
Lung				
Increased number of macrophages		19 %	63 %*	75 %
Influx of granulocytes (bronchi)	Moderate	0 %	25 %	38 %*
	Severe	0 %	0 %	0 %
Lung-associated lymph nodes				
Influx of granulocytes	Moderate	0 %	13 %	63 %**
	Severe	0 %	0 %	0 %

* $p < 0.05$; ** $p < 0.01$

1.1.4.2 Respiratory sensitisation in mice (Matheson et al., 2005a; Matheson et al., 2005b)

Study references:

[5] Fraction of animals for which the number of events with an increase in respiratory rate amounted to more than three times the standard deviation of the individual baseline (similar period during the pre-challenge phase), no significance testing reported.

[6] Fraction of animals for which the area under the (respiratory rate) curve exceeded three times the standard deviation of the individual baseline (similar period during the pre-challenge phase), no significance testing reported.

Matheson J.M., Johnson V.J., Vallyathan V., and Luster M.I. (2005b): Exposure and immunological determinants in a murine model for toluene diisocyanate (TDI) asthma. Toxicological Sciences 84 (1), 88-98. DOI: 10.1093/toxsci/kfi050 (last accessed 2016-09-19); Matheson J.M., Johnson V.J., and Luster M.I. (2005a): Immune mediators in a murine model for occupational asthma: Studies with toluene diisocyanate. Toxicological Sciences 84 (1), 99-109. DOI: 10.1093/toxsci/kfi051 (last accessed 2016-09-20)

The results of this study have been published in two publications of which only the main study (Matheson et al., 2005b) is summarised below, as (Matheson et al., 2005a) primarily addressed mechanistic questions which are not of relevance for this CLH dossier. Text, tables and figures are reproduced from the original publications, with slight editorial modifications by the DS.

Test substance

TDI (80:20 molar mixture of 2,4:2,6 isomers provided by Bayer, USA, Pittsburgh, PA)

Test animals

Preliminary studies were conducted using several mouse strains including C57BL/6, BALB/c, and B6C3F1 mice. Since the C57BL/6 strain produced the most robust responses under the current exposure conditions, the strain was used in the current studies. Female wild-type C57BL/6 J and FcErIg knockout (B6.129-FcerIg5tmlRav.N12) mice, deficient in the g chain of the FcerI, FcgRI, and FcgRIII genes, were obtained from Jackson Laboratory (Bar Harbor, ME), and Taconic (Germantown, NY), respectively, at approximately 5 to 6 weeks of age. Upon arrival the mice were quarantined for 2 weeks and acclimated to a 12-h light/dark cycle. Animals were housed in microisolator cages in pathogen-free and environmentally controlled conditions at NIOSH facilities in compliance with AAALAC approved guidelines and an approved IACUC protocol (03-JM-M-005). Food and water were provided ad libitum.

Methods

Atmosphere generation

TDI vapours were generated by passing dried air through an impinger that contained 3 mL TDI. A computer-interfaced mass flow controller (Aalborg Instruments, Orangeburg, NY, model GFC-37, 0–20 LPM) regulated the TDI concentration in the chamber, while a similar mass flow controller (model GGC-47, 0–100LPM) regulated the diluent air. Temperature and relative humidity were monitored by a Vaisala transmitter (Vaisala Inc., Woburn MA, type HP-233) interfacing with the TDI and diluent air controllers in a National Instruments (Austin TX) data acquisition/control system. The generation system produced TDI vapour, free of TDI aerosol.

Real-time monitoring of the chamber atmosphere was performed using an AutostepTM continuous toxic gas analyzer (Bacharach, Inc, Pittsburgh, PA) with TDI concentrations never varying more than 10 % in the study.

Induction regime

Mice were exposed to TDI by inhalation either of 20 ppb of TDI for 6 weeks, 5 days per week, 4 h per day (subchronic exposure), or of 500 ppb TDI for 2 h (acute exposure), in a 10 L inhalation chamber with only the heads of the animals extended into the chamber.

Challenge

Challenge (1 h, 20 ppb TDI) was performed on all groups 14 days following the last day of subchronic or acute exposure. The 6-week exposure period is the time during which sensitisation to TDI develops in the current models. Therefore, mice that were exposed to TDI during this 6-week period followed by challenged are, henceforth, referred to as ‘‘sensitised/challenged’’ groups.

Control groups

Three control groups were examined, including an air sensitised/air challenged, TDI sensitised/air challenged, and air sensitised/TDI challenged treatment group. As all control groups responded similarly, for convenience, only results from the air sensitised/TDI challenged control treatment are shown in the publication and are, henceforth, referred to as ‘‘controls’’ except in AHR studies, where values for all groups were reported.

Tissue collection

Groups of mice from each treatment group were sacrificed 48 h after airway challenge, using a CO_2 atmosphere, and lungs and nares were collected. Lungs were inflated with 10 % neutral buffered formalin (NBF), and tissues were immersed in 10 % NBF for 24 h, after which the nares were decalcified. The tissues were embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin for histopathological assessment. PAS staining was performed to identify goblet metaplasia and Chromatrope 2R/Mayer's Hematoxylin staining for eosinophil identification. The histopathological grading system was performed blinded and expressed on a 0–5 scale for each animal, with 0 representing no change, 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderate/severe, and 5 = severe.

Additional groups of mice were sacrificed 24 h after challenge and utilised for bronchoalveolar lavage fluid (BALF) and blood collection. To obtain BALF, mice were anaesthetised with 50 mg/kg of pentobarbital, exsanguinated, and intubated with a 20-gauge cannula positioned at the tracheal bifurcation. Each mouse lung was lavaged three times with 1.0 mL of sterile HBSS and pooled. BALF recovery was 80 ± 5 % for all animals. The BALF samples were centrifuged, and the supernatant frozen at -80 °C until enzyme analysis. The cells were resuspended at 105 cells/mL of HBSS, and 0.1 mL was used for cytospin preparations. The slides were fixed and stained with Diff-Quick (VWR, Pittsburgh, PA), and differential cell counts were obtained using light microscopic evaluation of 300 cells/slide. Total cell counts were performed with a haemocytometer. In replicate experiments, lungs were collected 24 h following challenge, and tissues were frozen in RNAlater (Qiagen, Valencia, CA) and stored at -80 °C for reverse transcriptionpolymerase chain reaction (RT-PCR) analysis. Tissues frozen in liquid nitrogen were incubated with RNAlaterICE (Ambion, Austin, TX) at -20 °C for 24 h prior to RNA isolation.

Transfer experiments

Adoptive and passive transfer experiments were conducted to assess the role of specific immunity in the asthma response. For adoptive transfer experiments, single cell suspensions were prepared from groups of mice exposed to TDI for six weeks or air sham controls by gently pressing pooled lymph nodes (mediastinal and auricular) and spleens through a stainless steel screen. The cell suspensions were washed with HBSS (Gibco, Grand Island, New York), the cell number adjusted to 2 x 10^7 cells/mL, and aliquots layered onto Lympholyte-M (Accurate Chemical, Westbury, NY).

After centrifugation at 2500 rpm, the lymphocyte interface was collected and washed, and 5.0 x 10^7 cells in 0.5 mL volumes were injected intravenously into naive recipients. B or T cell depletion was conducted by incubating isolated lymphoid cells with either panT or panB Dynabeads (Dynal Biotech Inc., Lake Success, NY) at a 7:1 cell:bead ratio, according to the manufacturer's instructions. The respective T and B cell populations were > 98 % pure, as assessed by FACS analysis on a FACS Calibur (BD Biosciences, Palo Alto, CA) utilising anti-CD3 and anti-B220 FITC conjugated monoclonal antibodies (PharMingen, San Diego, CA). The resulting T and B lymphocyte populations were injected intravenously into naive recipients at a concentration of 2.9 x 10^7 cells and 2.5 x 10^7 cells, respectively, in 0.5 mL volumes. To measure TDI-specific serum activity, naive mice received an intradermal injection of 30 mL heat-inactivated (56 °C, 4 h) or non-heated pooled serum into the dorsum of the right ear from either TDI sensitised/challenged mice or control mice. Animals were challenged 24 h later with 1 % TDI (in acetone:olive oil, 4:1) on the dorsum of the same ear, and the change in ear thickness was compared to the thickness pre-challenge. Additional groups of mice received an intravenous injection of 200 mL of either heated or unheated pooled sera from TDI sensitised/challenged or control mice. Twenty-four hours after intravenous lymphocyte or serum transfer, mice were challenged either by inhalation with 20 ppb TDI for 1 h or by a single application of 25 mL of 1 % TDI (in acetone:olive oil, 4:1) onto the dorsum of the right ear, as previously described (Ebino, 1999). Respiratory responses including pathology (as outlined above) and airway responsiveness to methacholine (see below) were determined 48 and 24 h following challenge, respectively. The ear challenge response was determined by measuring the change in ear thickness from baseline pre-challenge ear thickness 24 h following TDI application. Cell proliferation in the draining lymph node was determined in an additional group of recipient mice using a modification of the local lymph node assay, as originally described by (Dearman and Kimber, 2000). Twenty-four hours after challenge, the mice were injected intravenously with 200 mL of ^{3}H-thymidine (specific activity 0.1 mCi/mL; Amersham, Piscataway, NJ), and incorporation of ^{3}H-thymidine into DNA in the draining auricular lymph nodes was measured.

Antibody detection

Total serum IgE was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (Satoh et al., 1995). Briefly, plates were coated with 5 mg/mL of rat monoclonal antimouse IgE (PharMingen). Serial two-fold dilutions of test sera, starting at a 1:5 dilution, were added and incubated with peroxidase-goat anti-mouse IgE (1:1000, Nordic Immunological Laboratories, Capistrano Beach, CA) and developed with ABTS substrate, 2,20-azinobis(3-ethylbenzthiazoline-6-sulfonic acid). Total serum IgE concentrations were derived from a standard curve obtained using murine monoclonal anti-DNP IgE (Sigma, St. Louis, MO). TDI-specific antibodies were detected by ELISA using a TDI-mouse serum albumin conjugate, kindly provided by Dr. Meryl Karol (University of Pittsburgh, Pittsburgh, PA), as previously described (Satoh et al., 1995). Serial two-fold dilutions of test sera, starting at a 1:5 dilution, were added to individual wells and incubated with peroxidase-conjugated, goat anti-mouse antibodies against either total IgG (1:400, Sigma, St. Louis, MO), IgG_1, or IgG_{2a} (both at 1:400, The Binding Site, Birmingham, UK) and developed with ABTS substrate. Antibody titers were determined by plotting the serial dilution curve for each sample individually vs. the optical density (OD) for each dilution of that sample. A cut-off OD of 0.2 (average OD of challenge only mouse serum was 0.06 ± 0.005) was used to determine the titer.

Eosinophil peroxidase activity (EPO)

Measurement of EPO activity was performed on BALF supernatants according to the method of (Bell et al., 1996), with slight modifications. Briefly, 0.1 mL of peroxidase substrate solution, consisting of o-phenylenediamine dihydrochloride (OPD), urea hydrogen peroxide, and phosphate-citrate buffer (Sigma Fast Tablets, Sigma, St. Louis, MO), was added to 0.1 mL of the BALF supernatant. The mixture was incubated at 37 °C for 30 min before stopping the reaction with 50 M of 2 N hydrochloric acid. Optical densities were measured at 490nm (OD490). Non-specific activity was determined by treating duplicate sample sets with the EPO inhibitor, 3-amino-1,2,4-triazole (2 mM,Sigma), and was always less than 10 % of the non-treated samples. Results are expressed as OD490 corrected for background and volume of BALF supernatant retrieved (BALF recovery was 80 ± 5 %).

Airway hyperresponsiveness (AHR)

AHR to methacholine challenge was assessed, 24 h following TDI challenge, using a single chamber whole-body plethysmograph (Buxco, Troy, NY). A spontaneously breathing mouse was placed into the main chamber of the plethysmograph, and pressure differences between the main chamber and a reference chamber were recorded. AHR was expressed as enhanced pause (PenH), which correlates with measurement of airway resistance, impedance and intrapleural pressure and is derived from the formula:

$$PenH = [(Te – Tr)/Tr] \times Pef/Pif;$$

where Te = expiration time, Tr = relaxation time, Pef = peak expiratory flow, and Pif = peak inspiratory flow (Schwarze et al., 1999). Mice were placed into the plethysmograph and exposed for 3 min to nebulised PBS followed by 5 min of data collection to establish baseline values. This was followed by increasing concentrations of nebulised methacholine (0–50 mg contained in 1.0 mL of PBS) for 3 min per dose using an AeroSonic ultrasonic nebulizer (DeVilbiss, Somerset, PA). Recordings were taken for 5 min after each nebulisation. The PenH values during each 5 min sequence were averaged and expressed as percentage increase over baseline values following PBS exposure for each methacholine concentration.

Real-time RT-PCR

Tissues were homogenised, and total cellular RNA was extracted using the Qiagen RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. One microgram of RNA was reverse-transcribed using random hexamers and 60 U of Superscript II (Life Technologies, Grand Island, NY). Real-time PCR primer/probe sets for murine 18S, IFN_γ, IL-4, IL-5, and TNF_α were purchased as predeveloped kits from Applied Biosystems (Foster City, CA). Real-time PCR was performed using Taqman Universal Master mix with Amperase in an iCycler (Bio-Rad, Hercules, CA) for 1 cycle at 50 °C for 2 min (degrade carry over using Amperase), and 95 °C for 10 min, followed by 60 cycles at 95 °C for 15 sec and 60 °C for 1 min. The differences in mRNA expression between control and treatment groups were determined by the relative quantification method developed by (Pfaffl, 2001) utilising the threshold cycle (CT) method and real-time PCR efficiencies of the target gene normalized to the housekeeping gene 18S/rRNA.

Statistical analysis

All studies were replicated with representative data shown. For statistical analysis, standard one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test was used for multiple group comparisons. Student's two-tailed unpaired t test was used to determine the level of difference between two experimental groups, and $p < 0.05$ was considered a statistically significant difference. For the analyses of RT-PCR data, the fold change from the mean of the control group was calculated for each individual sample (including individual control samples to assess variability in this group centered around one) prior to ANOVA and SNK.

Results

AHR

The results with respect to Airway Hyperresponsiveness (AHR) are shown in Figure 1 below.

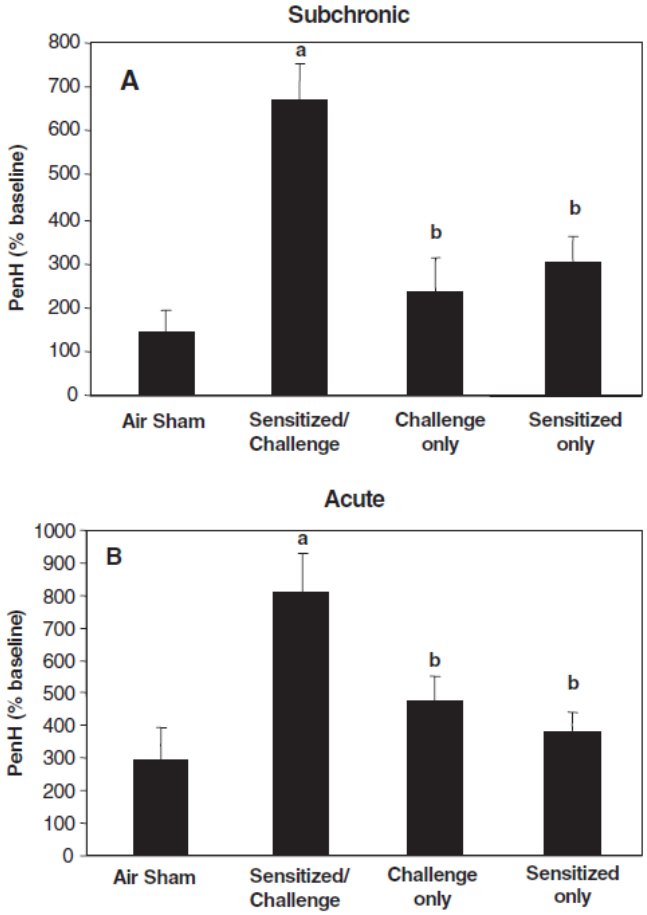

Figure 1: AHR in TDI-exposed mice. Mice which received air only, air sensitised/TDI challenged, TDI sensitised/air challenged, or TDI sensitised/challenged by either subchronic exposure (A) or acute exposure (B) were assessed for nonspecific methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine was determined 24 h after challenge and is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.48 ± 0.06) did not differ between treatment groups. Significantly different from a = air sham control group or b = sensitised/challenged group ($p < 0.05$, $n < 5$, mean ± SEM). Taken from (Matheson et al., 2005b).

Mice exposed to 20 ppb TDI by inhalation for 6 weeks and challenged 14 days later demonstrated a marked increase in AHR to methacholine. A slight increase in AHR to methacholine occurred in the sensitised-only and challenged-only groups, but was not statistically significant. Mice exposed to an acute high dose (500 ppb) of TDI followed 14 days later with 20 ppb challenge also exhibited significant AHR to methacholine challenge compared to controls. No differences in baseline PenH values were observed between treatment groups in the subchronic or acute exposure protocols. Furthermore, mice subchronically exposed to TDI show increased PenH values within 2 h following challenge with TDI, indicating TDI-specific airway responsiveness, an important characteristic of asthma.

For the reporting of the remaining parts of this study, the control group will represent mice that received air exposure for 6 weeks (subchronic) or 2 h (acute) followed by TDI challenge (challenge-only).

Antibodies

The results of the antibody assessment are shown in Figure 2.

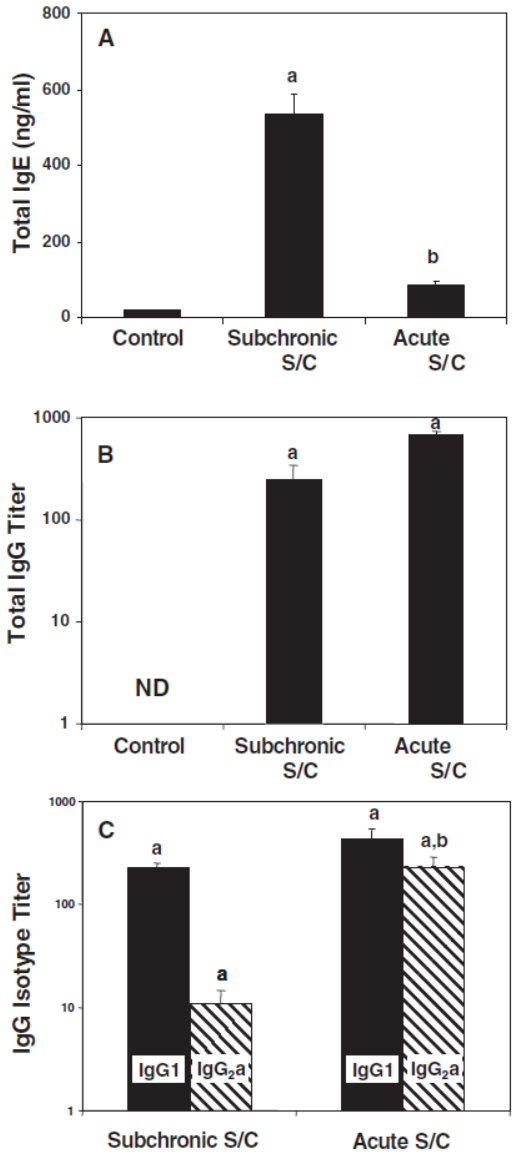

Figure 2: Total serum IgE levels and TDI-specific serum IgG antibody titers. Sera were collected 24 h after TDI challenge from mice that received TDI challenge only (control), subchronic low-dose TDI exposure, or acute high-dose TDI exposure. Total IgE levels (A), TDI-specific IgG antibodies (B), and TDI-specific IgG_1 and IgG_{2a} antibodies (C) are shown. No TDI-specific IgG antibodies were detected in the control group for (C). Significantly different from a = control group or b = subchronic sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). ND = not detected. From (Matheson et al., 2005b).

Twenty-four hours after TDI challenge, blood was collected from control and exposed mice and the serum analysed for total IgE and TDI-specific IgG antibodies. Total serum IgE levels in mice that received subchronic TDI exposure were increased by approximately 10-fold compared to control mice, while IgE levels in serum from mice that received an acute exposure to TDI were comparable to controls. Total IgG TDI-specific antibodies, as well as IgG_1 and IgG_{2a} TDI-specific antibodies, were consistently detected and significantly elevated in both the subchronic low-dose and the acute high-dose exposed groups, compared to undetectable levels found in the control group. In addition, while there were equivalent levels of IgG_1 and IgG_{2a} antibodies

in the acute high-dose group, IgG_1-specific antibodies were at least 30-fold higher than IgG_{2a} antibody levels, in subchronically exposed mice. IgG_1 and IgG_{2a} antibodies specific for TDI were not detectable in sera of control mice (not shown).

Markers of inflammation

The pathological changes induced by TDI exposure are summarised in Table 10, followed by an overview of the findings from BALF analysis in Figure 3.

Table 10: Summary of pathological changes induced by TDO exposure, from (Matheson et al., 2005b). Histopathological changes were assessed 48 h after the last TDI inhalation challenge. Values are expressed on a 0–5 scale, with 0 representing no changes, 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately/severe, and 5 = severe. Mean individual severity within a group was calculated by added severity scores of all animals and then dividing that by the total number of animals. a = Significantly different from control group ($p < 0.05$). b= Epithelial changes represent epithelial hyperplasia, epithelial regeneration, and loss of structure. * = Mean ± SEM (n = 5).

Tissue alteration		Control	Subchronic	Acute
Nares				
Exudate		0.2 ± 2*	2.5 ± 2[a]	2.2 ± 6[a]
Goblet metaplasia		1.2 ± 0.2	4.2 ± 0.1[a]	4.3 ± 0.2[a]
Inflammation	**Lymphocytes**	0.5 ± 0.2	2.2 ± 0.4[a]	0.5 ± 0.3
	Neutrophils	0.8 ± 0.2	2.7 ± 0.5[a]	1.8 ± 0.6
	Eosinophils	0.4 ± 0.3	2.9 ± 0.5[a]	0.7 ± 0.3
	Epithelial changes	0.2 ± 0.2	2.1 ± 0.1[a]	3.3 ± 0.1[a]
	Hyaline droplet	0.2 ± 0.3	3.1 ± 0.4[a]	2.0 ± 0.2[a]
Lung				
Goblet metaplasia		0	1.9 ± 0.3	2.3 ± 0.7[a]
Inflammation	**Lymphocytes**	0.7 ± 0.3	3.3 ± 0.4[a]	0.8 ± 0.3
	Neutrophils	0	1.9 ± 0.3[a]	0.2 ± 0.2
	Eosinophils	0	3.4 ± 0.3[a]	0.2 ± 0.1
	Macrophages	0	2.4 ± 0.3[a]	1.7 ± 0.2[a]
	Epithelial changes	0	2.4 ± 0.4[a]	1.2 ± 0.3[a]

Airway inflammation is a central feature of the asthmatic response to TDI and is considered a key manifestation of underlying bronchial hyperresponsiveness. Mice subjected to the subchronic TDI exposure regimen presented histological changes in the lungs and nares consistent with an inflammatory response, manifested by neutrophil, lymphocyte, eosinophil, and macrophage infiltration. Tissues at these sites exhibited degenerative cellular changes including loss of cilia, goblet cell metaplasia, septal exudate, hyaline droplet formation, and epithelial hyperplasia. Mice exposed by the acute high-dose exposure regimen exhibited similar histopathology as observed in the subchronic exposure, but fewer inflammatory cells, including eosinophils. Control mice revealed minimal histopathological changes that were contained primarily in the nares.

Total cell numbers in the BALF of mice exposed following the subchronic protocol were increased two-fold compared to the control group. Differential analysis showed that large increases in eosinophils and lymphocytes were responsible for the observed increase in cell recruitment. There was also a significant increase in neutrophil infiltration into the lung, although to a much lesser extent than other inflammatory cells. Macrophages were the predominant cell type in the lung of control mice, representing over 95 % of the cells, whereas macrophages decreased to 56 % of the total cell population in the subchronically exposed mice following challenge. Mice exposed to the acute high-dose treatment exhibited an 8-fold increase in lymphocyte numbers following challenge, but minimal effects on other inflammatory cells, including eosinophils. Corresponding to the increase in eosinophil numbers, EPO activity in BALF supernatants was significantly elevated in subchronically exposed mice after challenge, while no increase in activity was found in the acute high-dose treated animals.

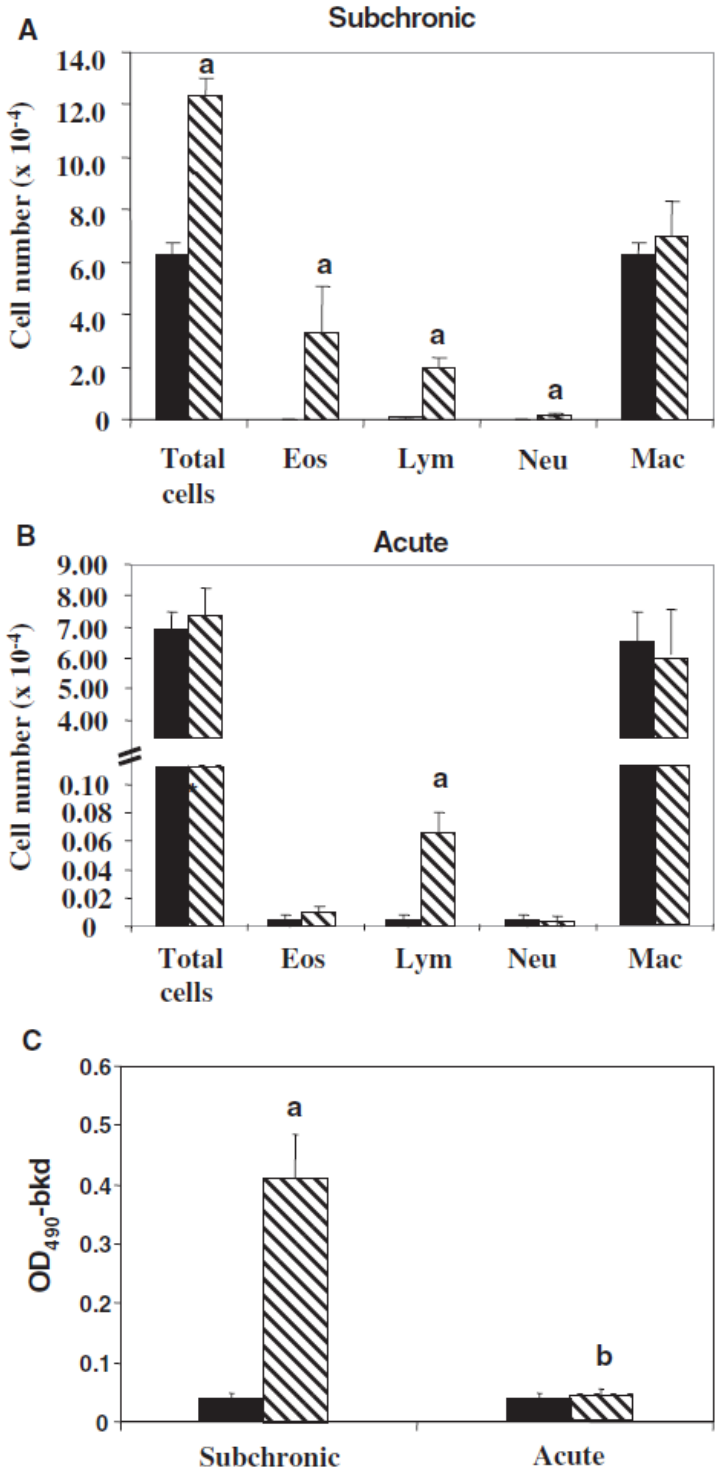

Figure 3: Cellular distribution and EPO activity in bronchoalveolar lavage fluid (BALF). BALF was collected 24 h after TDI challenge, and cytospin preparations were examined for cellular content. Differential cell counts for subchronically exposed mice (A) and acutely exposed mice (B) were determined using light microscopy by evaluation of 300 cells per slide. Data are presented as total cell number for each population in the BALF (Eos = eosinophil; Lym = lymphocyte; Neu = neutrophil; Mac = macrophage). BALF supernatants were measured for eosinophil peroxidase activity (C), and the data are expressed as the optical density at 490 nm after background subtraction (OD490 – bkd). Solid bars represent control group responses, and stripped bars represent TDI sensitised/challenged group responses. Significantly different from a = control group or b = subchronic sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). Taken from (Matheson et al., 2005b).

Cytokines have been implicated in the recruitment of inflammatory cells to the lung and in the pathogenesis of asthma. To determine the effects of TDI on the relative expression of cytokines in the airway, RNA was isolated from the lungs of mice 24 h after challenge, and the levels of IL-4, IL-5, TNF_{α} and IFN_{γ} mRNA were determined by real-time PCR, cf. Figure 4.

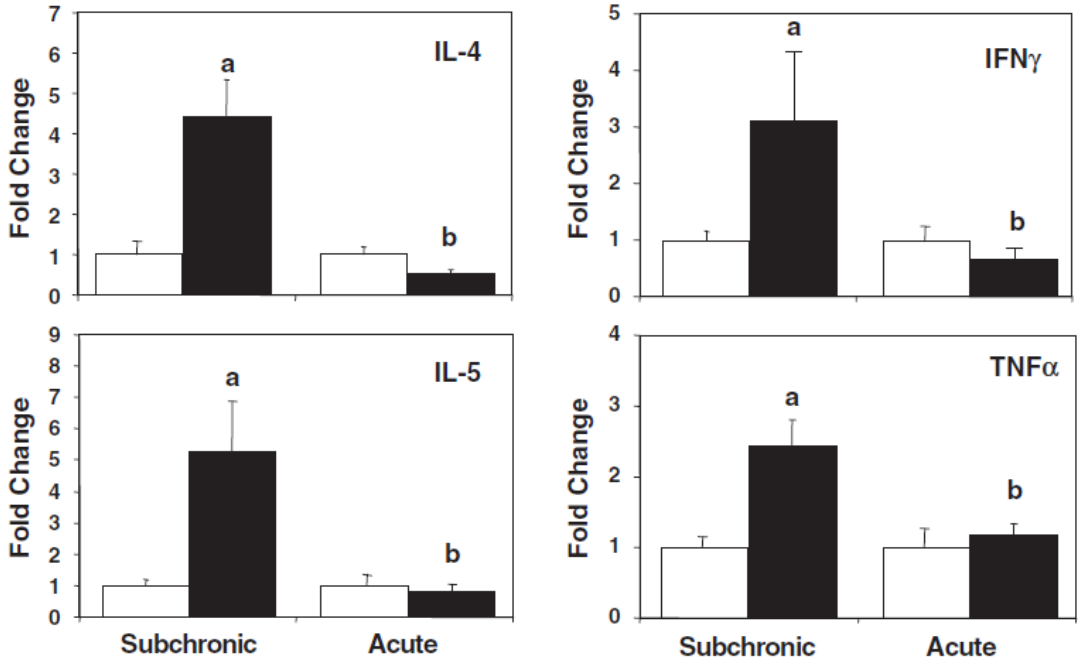

Figure 4: Inflammatory cytokine gene expression in the lungs of TDI-exposed mice. Twenty-four hours following challenge, RNA was isolated from lungs and real-time RT-PCR was performed using IL-4, IL-5, IFN_{γ}, TNF_{α}, or 18s (internal control)-specific primer/probe sets. Cytokine mRNA expression data for subchronic and acute exposure mice are presented as fold change from the respective control group. Open bars represent control group responses, and solid bars represent TDI sensitised/challenged group responses. Significantly different from a = control group or b = subchronic sensitised/challenged group, ($p < 0.05$, n = 4, mean ± SEM).

Compared to the control group, subchronic TDI-exposed mice showed significant elevations in IL-4, IL-5, IFN_{γ} and TNF_{α} mRNA transcripts following TDI challenge. In contrast, no increase in expression of IL-4, IL-5, IFN_{γ} or TNF_{α} was observed in the lungs of mice that received acute TDI exposure.

Transfer experiments

To determine whether specific immunity was involved in the asthmatic response to TDI, adoptive transfer experiments were conducted in which lymphocytes, B cells, or T cells from TDI-exposed mice were transferred into naive recipients. Twenty-four hours following cell transfer, the mice were challenged with 20 ppb TDI, and lung inflammation and airway reactivity were assessed 48 and 24 h later, respectively.

Histological examination of lungs from mice that received lymphocytes from subchronic TDI exposed animals showed slight, diffuse infiltration of lymphocytes and eosinophils following TDI challenge, while those receiving lymphocytes for acute TDI exposed group revealed lymphocyte infiltration but no eosinophils. No lung inflammation was evident after challenge in transfer mice that received lymphocytes from control animals. Naive mice that received either purified lymphocytes, T cells, or B cells from mice that underwent subchronic exposure also displayed significantly increased responsiveness to methacholine 24 h following TDI challenge, when compared to the control group. Recipient mice that received unfractionated lymphocytes from mice in the acute treatment group also showed a significant increase in AHR to methacholine 24 h following TDI challenge, although the magnitude of increase over the control group was about half that observed following total cell transfer from subchronic exposure mice. Adoptive transfer experiments with purified B and T cells from mice that received the acute exposure regimen were not conducted (Figure 5).

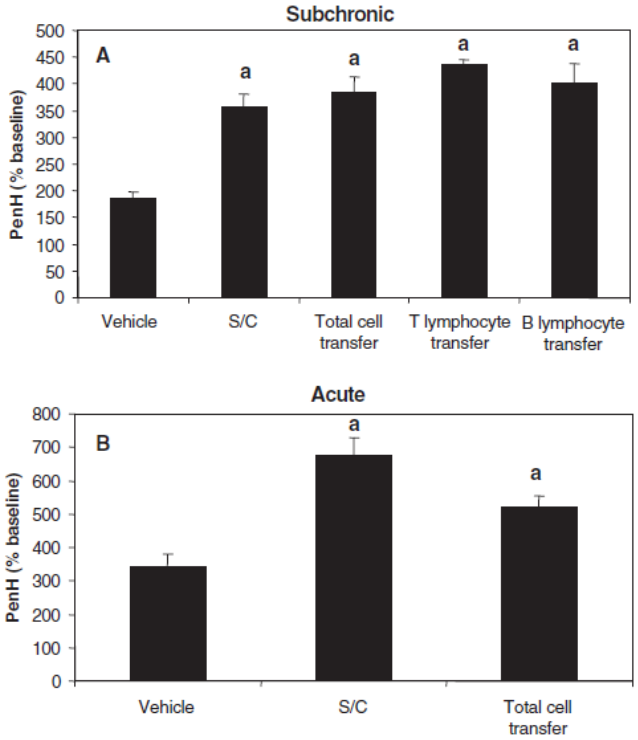

Figure 5: AHR following adoptive transfer with lymphocytes from TDI-exposed mice. Lymphocytes pooled from the auricular lymph nodes and spleens from TDI-subchronically exposed (A) or acutely exposed mice (B) were injected i.v. into naive recipient mice that where challenged by TDI inhalation 24 h later. Twenty-four hours following TDI challenge, mice which received vehicle, total lymphocytes, T lymphocytes, or B lymphocytes, as well as a TDI-exposed positive control group (sensitised/challenged, S/C) were assessed for methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.51 ± 0.07) did not differ between treatment groups. a = Significantly different from vehicle control group, $p < 0.05$, n = 5, mean ± SEM. From (Matheson et al., 2005b).

To help determine whether TDI-specific lymphocytes were present in the transfer experiments, lymphocytes from mice that underwent subchronic TDI exposure were adoptively transferred to naive recipients, and 24 h later the recipients were challenged with 25 mL of 1 % TDI on the dorsum of the ear. Ear swelling was determined following an additional 24 h. Mice that received unfractionated lymphocytes, B cells, or T cells produced a significant ear swelling response following TDI challenge. Cell proliferation in the draining auricular lymph node was also significantly increased in adoptively transferred mice following TDI ear challenge, although the response following transfer of B cells was minimal compared to T cells. This was evidenced by 20-fold, 8-fold, and 2.4-fold increases in ^{3}H-thymidine uptake in mice receiving total lymphocytes, T lymphocytes, and B lymphocytes, respectively, compared to controls. Transfer of lymphocytes from acutely exposed mice was not performed in these experiments (Figure 6).

To help elucidate the role of humoral immunity in TDI-induced asthma, passive transfer experiments were performed in which serum from mice that had been exposed subchronically and challenged with TDI was administered to naive mice. Histological examination of lungs from mice that received serum from TDI-exposed animals showed minimal diffuse infiltration of lymphocytes and eosinophils 48 h after TDI challenge. No lung inflammation was evident after challenge in transfer mice that were injected with serum from control animals. Twenty-four hours following serum transfer, mice were challenged with TDI by inhalation, and AHR to methacholine was assessed 24 h later. Mice that received non-heated serum from subchronically exposed mice displayed increased AHR to methacholine challenge (50 mg/mL) at 24 h after TDI challenge. Heat inactivation of the serum (56 °C, 4 h), which destroys IgE activity, removed the ability to transfer AHR. Mice injected intradermally with sera (30 mL) from subchronically exposed mice and challenged 24 h later with 1 % TDI demonstrated a dermal response, measured as an increase in ear thickness. Heat inactivation of the sera also markedly, but not completely, reduced the dermal response, possibly reflecting the presence of other soluble mediators in the serum that are heat-stable (Figure 7).

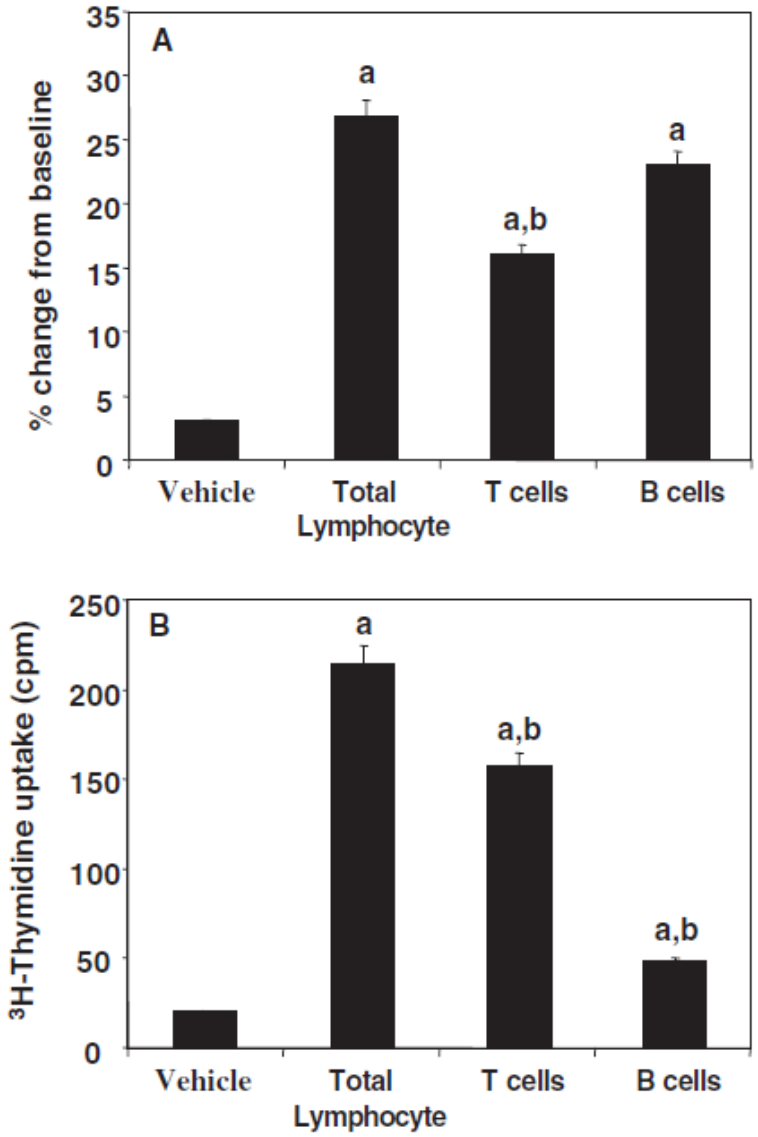

Figure 6: Contact hypersensitivity to TDI following adoptive transfer of lymphocytes from mice subchronically exposed to TDI. Lymphocytes pooled from the auricular lymph nodes and spleens from TDI-exposed mice were injected i.v. into naive recipient mice. Mice were challenged 24 h later with 1 % TDI on the dorsum of the right ear, and after an additional 24 h, contact hypersensitivity responses were measured as a function of challenge-induced increases in ear thickness (A) and ^{3}H-thymidine uptake in the draining auricular lymph nodes (B). Significantly different from a = vehicle control group or b = total lymphocyte transfer group, ($p = 0.05$, $n = 4$, mean ± SEM). From (Matheson et al., 2005b).

The role of antibody in TDI-induced asthma was further explored using FcErIg transgenic mice, which lack the g chain subunit of the FceRI, FcgRIII, and FcgRI receptors and, thus, do not mount functional IgG and IgE immune responses. Transgenic mice were exposed to TDI by subchronic inhalation, and methacholine reactivity was assessed at 24 h following TDI challenge. Increased AHR in transgenic mice was similar to the controls. Changes in lung cytokine mRNA expression were also examined in FcErIg transgenic mice. In contrast to the sensitized/challenged wildtype group, the levels of the asthma-associated cytokines IL-4, IL-5, IFN_g and TNF_a in the subchronically exposed FcErIg transgenic mice were not increased (Figure 8).

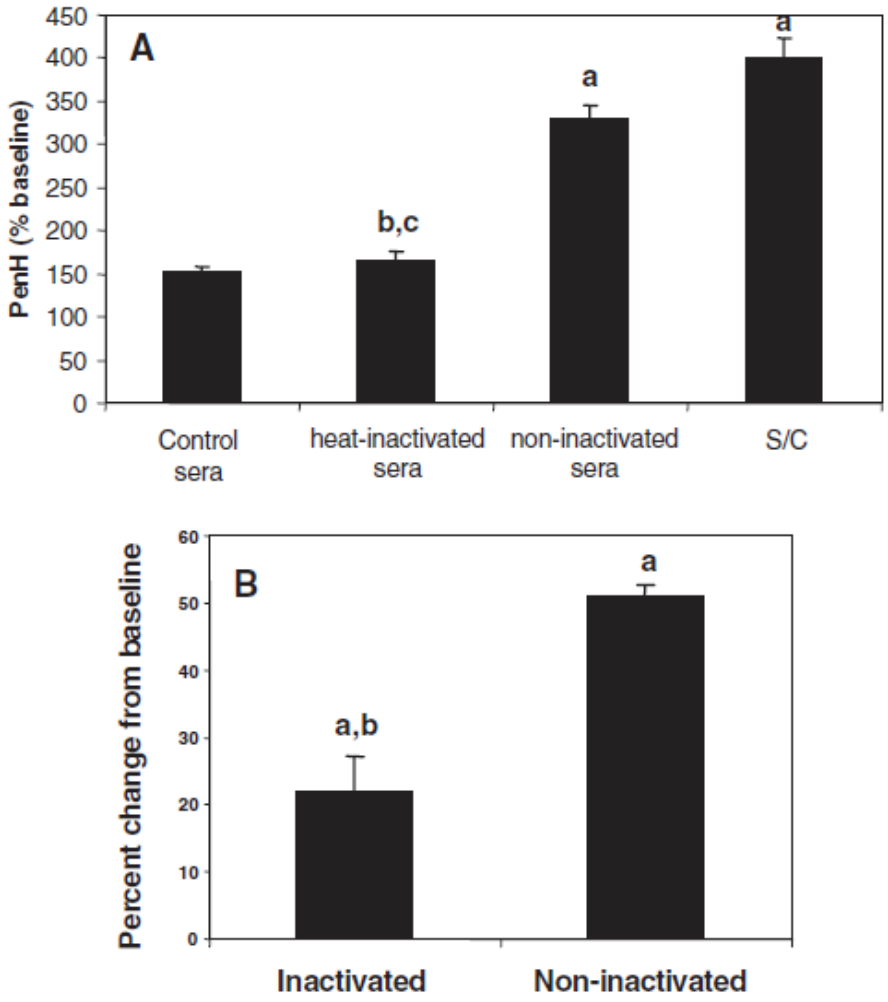

Figure 7: AHR following passive transfer of TDI immune serum. Sera pooled from TDI subchronically exposed mice was injected i.v. into naive recipient mice. (A) Twenty-four hours later mice were challenged with TDI (20 ppb via inhalation route for 1 h) and 24 h post-inhalation challenge, mice which received control sera, heat-inactivated TDI sera, noninactivated TDI sera, or TDI subchronic sensitised/challenged (S/C, positive control) were assessed for methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.45 ± 0.04) did not differ between treatment groups. (B) Heat-inactivated or non-inactivated pooled serum from TDI subchronically exposed mice was injected intradermally into the dorsum of the right ear of naive recipient mice. Twenty-four hours following transfer, mice were challenged with 1 % TDI on the same ear, and responses were measured as a function of challenge-induced increases in ear thickness 24 h post-challenge. Data are presented as percent change from pre-challenge ear thickness of the right ear. Significantly different from a = control serum treated group, b = non-inactivated treated serum group, or c = subchronic sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). The response to control sera was compared to that of normal mouse sera, and no difference was observed (data not shown). From (Matheson et al., 2005b).

Conclusion of the authors

In conclusion, a mouse model is described that demonstrates low-level subchronic TDI inhalation induces pathology, consistent with allergic asthma, manifested by airway inflammation, lung eosinophilia, increased AHR, asthma associated histopathology, Th cytokine expression, elevated serum IgE, and TDI-specific antibodies. Asthmatic symptoms also occur following high-dose, acute exposure, but the response is less robust, failing to demonstrate eosinophilia, elevated serum IgE levels, or Th cytokines. Evidence is also presented that, like allergic asthma, TDI asthma following subchronic exposure, while associated with a T_H2 response involving IgE antibodies, also involves T_H1 responses.

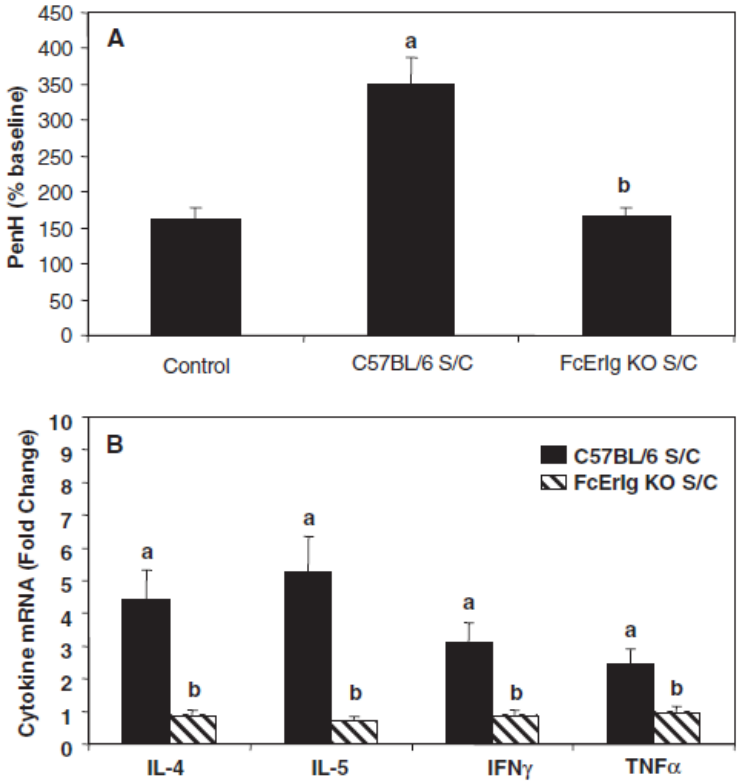

Figure 8: AHR and lung cytokine expression in mice lacking Fc-e and Fc-g (FcERIg) receptors after subchronic exposure to TDI. (A) Twenty-four hours following TDI inhalation challenge, control mice, FcERIg knockout S/C mice, or TDI-subchronically exposed C57BL/6 S/C mice were assessed for methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine was determined 24 h after challenge and is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.42 ± 0.08) did not differ between treatment groups. (B) Twenty-four hours following TDI challenge, mice were sacrificed, RNA was isolated from the lungs, and real-time RT-PCR was performed using IL-4, IL-5, IFN-γ, TNFα, and 18S-specific primer/probe sets. Data are presented as fold changes from the corresponding control strain. Significantly different from a = control group or b = wild-type sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). S/C = TDI sensitised/ challenged C57BL/6 mice from subchronic exposure. From (Matheson et al., 2005b).

1.1.4.3 Hoymann et al., 1995

Summary as provided by the lead registrant for MDI (the full study report was not available to the DS).

Study reference:

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

Only a IUCLID summary of this study was available from which only the details relevant for RS are reproduced below. Details are confined to findings.

Test type:

Combined chronic/carcinogenicity test claimed to be similar to OECD 453, but with only female animals exposed and exposure limited to 17 h/d. GLP claimed.

Test substance:

Monomeric 4,4'-methylenediphenyl diisocyanate (Desmodur 44 M Schuppen from Bayer AG, Leverkusen); 13 batches were tested (purity: > 99.5 %)

Test animals:

Rat, Crl:[WI]BR Wistar, female. At the start of the study the animals were approximately 10 weeks old. Acclimation: approx. 2 weeks. Origin: Charles River Wiga GmbH, Sulzfeld. 80 females per dose; at each dose level there were additional 80 rats per group in satellite groups for:

- chronic toxicity over 12 months (20 animals),
- lung function over 20 months (12 animals),
- lung clearance over 20 months (8 animals),
- bronchoalveolar lavage, biochemistry over 3 months + 1 week recovery (20 animals), and
- bronchoalveolar lavage, biochemistry over 12 months + 1 week recovery (20 animals).

Administration/exposure:

Choice of the exposure concentrations was done after a range-finding test (90-day study at 0.3, 1 und 3 mg/m³, under exposure regime of ca. 18 hours/day, 5 days/week), where a no observed effect concentration was derived (NOEC: 0.3 mg/m^3), based on substance-related effects seen in the highest and to some extent also in the mid-dose group. MDI aerosol was generated using an evaporation-condensation technique. The rats were exposed via whole-body exposure to concentrations of 0-0.2-0.7-2.1 mg/m^3, 17 h/d, 5 d/wk, for up to two years in 6 m^3 stainless steel inhalation chambers (horizontal air flow, renewal rate: approx. 15-fold per hour). Since the vapour saturation of MDI at 23 °C is about 0.1 mg/m^3, a part of the exposure was as vapour. Monitoring of total MDI was performed by gravimetrically calibrated, light scattering aerosol sensors. Concentrations of monomeric MDI in the inhalation chamber were measured with HPLC. The median mass aerodynamic diameters (in µm) were 1.03, 1.03, and 1.06, respectively. Controls: yes, sham-exposed.

Examinations:

Clinical signs:

All animals were observed for clinical signs at least once a day; if clinical signs were present, the animals were further examined; animals in bad condition were killed and organs put in formalin.

Organs examined at necropsy:

Macroscopic examination: full pathological examination is done on the surviving rats of the chronic tox test killed at 12 months exposure (satellite groups) and at 12 months resp. 24 months (animals with number 101-120 resp. 1-80) of the carcinogen test. Following organs are preserved in 10 % neutral buffered formalin solution: all organs/tissues that are macroscopically changed, brains, pituitary, thyroid, thymus, larynx and laryngopharynx, trachea, lungs, heart, aorta, pancreas, liver, kidney, adrenals, periferal nerve, sternum, femur and knee, vertebrae, tongue, lymph nodes (submandibular and mesenteric), mediastinal lymph nodes, nose, sinus, eyes/Harderian glands; lacrimal glands (extraorbitale), ovaries, uterus and vagina, mammary, skin, oesophgus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, muscles, pancreas, mesenterium. Lungs (incl trachea), under +/- 20 cm water pressure, are preserved in formaline solution.

Organ weights: are performed on the animals of the satellite group used for chronic tox test after 12 months of exposure: in 10 animals/ group: fresh weights of brain, liver, kidneys and adrenals and ovaries. Also the relative organ weights are calculated (vs. the body weight at the end of the test). This examination was not performed in rats after 24 months of testing due to increased mortality and the number of surviving animals being too limited to allow any firm conclusions to be drawn. In the satellite groups used to examine BAL (10 animals/ group) at the end of the exposure time as well as on the remaining 10 animals/group after recovery (=after 20 months: in surviving animals of the 20 animals/group at end of the test) terminal body weights and fresh weight on lungs (incl trachea) as well as the relative lung weight are calculated.

Microscopic examination (light microscopy) was done for all animals of the control group and the high dose group of the carcinogenicity test and the chronic tox after 12 months, on above tissues/organs after haematoxylin-eosin staining (Lilly-Meyer). In case of substance related pathological findings found in these groups, all corresponding organs (respiratory tract) of all other animals of low and mid-dose groups are examined. Moreover all organs with tumor-like or similar modifications were histologically examined. Peer review of the lung examinations (review examination by an external pathologist by Prof. Dr. D.L. Dungworth,

University of California, Davis, USA. Data record and statistical treatment of the pathological findings was done using the PLACES program.

Other examinations:

- lung function: on rats under narcosis, with non-invasive method. After 6, 12 and 17 months identical tests were done on the same rats (of the satellite groups). a) Whole-body plethysmography and parameter on spontaneous breathing. b) Forced Expiration c) Lung volume and elasticity d) N-exchange test: homogenity of ventilation e) CO-diffusion test: diffusion,
- bronchoalveolar lavage (BAL): Biochemical and cytological parameter of lung lavage, b) measurement of surface tension,
- lung clearance, and
- investigations on MDI-metabolism: in blood and urine.

Statistics:

Differences between test and control groups are judged statistically significant at level $p<0.05$. Body weight and food consumption, absolute and relative organ weight and hematological/biochemical data, BAL, clearance and lung function data are checked for difference between groups by variance analysis. If statistical difference was found between group means, the mean of the test group was compared to the mean of the control by t-test (lung function) or adapted t-test (Dunnett-test). The Wilcoxon test was used for surfactant data. Qualitative and semi-quantitative data (histopathology) are analysed by Fisher-test.

Any other information on materials and methods incl. tables:

The photometrically determined chamber concentrations were 0.23, 0.70 and 2.05 mg/m^3, with standard deviations of 0.06, 0.17 and 0.37 mg/m³, respectively. The fraction of the total MDI concentration present as monomeric 4,4'-MDI was 43 %, 79 % and 85 %, respectively, for the low, mid and high exposure groups. The fraction of the total MDI concentration present as monomeric 4,4'-MDI was 43 %, 79 %, and 8 %, respectively, for the low, mid and high exposure groups. The fraction of the total MDI concentration present as monomeric 4,4'-MDI was 43 %, 79 %, and 85 %, respectively, for the low, mid and high exposure groups.

Results and discussion:

Mortality: decreased survival time was seen in all groups (including controls). This was due to the earlier onset of age related changes e.g. tumours of pituitary and mammary gland. The cause of this finding could not be foreseen at the start of the test nor can it be clarified. In the carcinogenicity test: No significant differences occurred between the test groups and the controls. After 17-18 months exposure (i.e. 19-20 months age) cumulative mortality was 50 %. Compared to internal and external historical data (1984-1988) on the same rat species, this represents a real decrease in survival time. After 17 months of exposure the weight differences from low, mid and high dose groups compared to controls were -6.7 %; -7.9 % and -11.3 %. However it should be noted that at day 0 the weights of mid and high dose group were 2.4 and 2.2 % lower.

Body weight: since 4.5 months of testing, the mean weight of the animals in the mid- and low dose groups were significantly decreased compared to the control group.

Organ weights: Lungs: relative fresh weights (normalised to body weight) for lungs are increased after 3, 12 and 20 months exposure. After 3 months: significantly increased weights in all test groups. After 12 and 20 months these differences are only present at the highest dose group. After 1 week recovery (clean air) following 3 months exposure, a recovery effect is seen in the low and mid dose. However, in the high dose group animals the lung weight remains sign increased. Histopathological changes corroborate with this finding. Other organs: no significant difference are seen between the test and control groups

Gross pathology: with exception of the changes as described under histopathological changes, no substance related changes could be found

Histopathology: I. After 12 months of exposure (satellite-groups): Non-neoplastic changes: Exposure related pathological changes were only found in the nose, lungs and lung associated lymph nodes (LALN). Nose:

Very low to low graded (multi)focal degeneration of the olfactory epithelium: in 5/15 animals of the high dose group; in 1/19 animals of the mid dose group. These changes were absent in the low and control group. Statistically different were control and high dose group. Other changes were seen but these were not statistically significant from the controls. After 12 months MDI exposure: MDA-DNA adducts were found in olfactory nose epithelium, however only in marginal amount. Remark: The proof of MDA-DNA adducts is possibly feigned by the strong protein binding. The toxicological relevance of this finding is doubtful since MDI leads only in high concentrations to degeneration of the olfactory epithelium (Greim H (ed.) 2008, in: Occupational Toxicants - Critical data evaluation for MAK values and classification of carcinogens, Wiley-VCH, Weinheim, Vol. 14). Lungs: Statistically significant multifocal to diffuse interstitial (septal) fibrosis in all exposure groups. Slight to moderate interstitial fibrosis in mid and high dose group: present in resp. 18/19 animals and 15/15 animals (diff. not statistically significant). In the low dose group: 6/19. Moderate (multi)focal bronchiole-alveolar hyperplasia: higher frequency in mid and high dose groups. Focal alveolar hyperplasia (Type II cells especially): only in exposed groups (1 animal in low and in mid dose; 3 in the high dose). Not significant different but presumably related to exposure. Alveolar accumulation of macrophages with inclusion of particles in low amount and dose related frequency: only present in groups exposed to the test substance (statistically different compared to control: low dose: 8/19; mid: 16/19 and high dose: 15/15 animals). Epithelium associated giant cells of Langhans: difference very significant in mid and high dose groups. Low to moderate interstitial mononuclear cell infiltration in control to high dose animals: resp. 2/18; 5/19; 18/19 and 13/15. In the BAL there were after 3 and 12 months in the highest dose; increased macrophages, lymphocytes numbers; after 20 months increased number of lymphocytes. At no point in time was there a change in the number of granulocytes. Lung associated lymph nodes (LALN): Exposure related multifocal accumulation of particle bearing macrophages: in the mid (16/19) and high (6/14) dose group (statistically different from control). Slight reactive hyperplasia of the lymphoid tissue associated with macrophage accumulation: dose dependent increase in incidence. Other organs: Exposure related changes could not be detected.

Histopathology: II. After 24 months of exposure (carcinogenicity test): Lungs: A dose related neoplastic effect was only seen in the lungs. In 1 animal of the high dose group: bronchiole-alveolar adenoma built of dysplastic alveolar cells (type II pneumocytes). Further: dose dependent (multi)focal high grade dysplastic alveolar hyperplasia. Exposure related changes could only be found in the nose, larynx, lungs and lung-associated lymph nodes. Nose (only examined in control and high dose group): (Multi)focal, in general moderate squamous metaplasia, mainly in the proximity of the olfactory epithelium (in high dose significantly higher than in control: 16/80 vs 5/80). (Multi)focal generally moderate Becker cell hyperplasia (50/80 vs 33/80) and inflammatory cell infiltration of the mucosa (29/80 vs 10/80). Other changes, non significant but obviously dose related were: metaplasia of the respiratory epithelium, degeneration, erosion, respiratory and/or olfactory epithelium. Larynx (only examined in controls and high dose group): Slight multi(focal) squamous metaplasia significantly higher (13/79 vs 1/80). Focal hyperkeratosis (in the area of the epiglottis) and inflammatory infiltration of the mucosa (however non significant). Lungs: Alveolar cell hyperplasia: in frequency and severity significant difference between mid and high dose compared to controls. In the following incidences and severity are described for the 3 dose groups (number of animals with grade of the effect: very slight, slight, moderate, high; total animals displaying these changes): Low dose: 1/80; 4/80; 2/80; 1/80; 8/80, Mid dose: 0/80; 5/80; 5/80; 2/80; 12/80, High dose: 0/80; 6/80; 8/80; 7/80; 21/80. Alveolar bronchiolisation: (Multi)focal bronchiole-alveolar hyperplasia: is significantly higher in mid and high dose group (frequency in low; mid, high dose and control: 3/80; 14/80; 41/80; 3/80). The grading of this finding appeared to be dose related. The moderate and high grade hyperplasia only occurred in resp 5 and 2 animals of the high dose exclusively. Interstitial and peribronchiolar fibrosis: In all MDI exposed groups: statistically highly ($p<0.001$) significant compared to control (low, mid, high dose; control: 51/80; 73/80; 77/80; 4/80). Also the severity was significant difference in the different exposure groups: generally very slight (minimal) in low dose; mainly slight and slight to moderate in the high dose group. Other statistically significant dose dependent effects in lungs: Focal to multifocal alveolar accumulations of particle-laden (MDI?) macrophages: in very slight to moderate grade in all exposure groups: 52/80; 70/80 and 78/80 (highly sign diff with controls). Identity of the inclusion could not be defined via light microscopy.

In BAL: after 3 and 12 months of exposure increased number of macrophages and lymphocytes were seen; after 20 months only increased number of lymphocytes. Interstitial mononuclear cell infiltration (mainly low grade): Statistically significant in all exposure groups: number of animals with this finding in resp low; mid,

high dose and controls were: 24/80; 48/80; 73/80 and 11/80. Accumulation of hemosiderin pigmented macrophages: from low to high grade dose dependent significantly increased in all exposure groups compared to controls: numbers for low, mid, high dose and control: 6/80; 9/80; 14/80 and 0/80. Small focal to multifocal cholesterol granulomas: in the high dose group: 11/80 vs 0/80 in controls. In the other groups: 4/80 low dose and 1/80 in the mid dose group. Focal osseous metaplasias: Incidence: significantly higher in high dose group vs control (resp. 11/80 and 1/80). In the low and mid dose group resp: 6/80 and 4/80. Lung associated lymph nodes (LALN; only examined in control and high dose group): Accumulation of macrophages with cytoplasmatic inclusions were seen in 68/80 high dose animals (highly significant differences with control were no such changes were observed). In addition, slight to moderate reactive lymphoid hyperplasia was seen, more frequent in high dose (13/80 vs control 6/80). Other organs: Exposure related changes could not be detected. Lung function tests: 1. Significant increased flow resistance in the small, peripheral air tracts in highest dose after 6 months. After 12 and 17 months also detected in the mid and low dose detected (cfr FEV0.1; FEF50 and FEF25). 2. Significantly reduced vital to total lung volume and elasticity of the lung tissue in the high dose already after 6 months (restrictive lung changes). After 12 resp 17 months increased incidence and finally also in the mid dose group and marginally in the low dose group. 3. Positive N-exchange test (indication of increased non-homogenity of the alveolar respiration) after 17 months in the mid and more expressed in the high dose group (already as a trend to be seen after 12 months). 4. Positive CO-diffusion test after 12 and 17 months : particularly in the high dose, less in the mid and marginally in the low dose group (indicating impairment of the diffusion through the alveolar-capillary membrane).

BAL findings: Changes in biochemical lavage parameters (increased lactate dehydrogenase, beta-glucuronidase, total protein, gamma-glutamyl transferase, hydroxyproline concentration, phospholipid concentration; indications of damage to the cell membrane vessel endothelium, cell necrosis, increased collagen metabolism) occurred generally already after 3 months exposure and increased after 12 and 20 months. After 1 week recovery with clean air, these findings seemed partially reversible. Increased concentration of surfactant-phospholipid were found in the mid and high dose groups. Functionally: a slight decrease in 'specific' surface activity of the phospholipid standardised surfact sample is observed in the high dose group (increased surface tension as measured by surfactometer). Increased lymphocyte concentration was seen after 3, 12 and 20 months (partially reversible after 1 week recovery with clean air). Increased number of macrophages after 3 months. The increased lung weights especially in the high dose group were still increased after 1 week recovery. This indicates chronic lung changes that were confirmed by the histopathological findings. Examination of the lung clearance (alveolar lung wash): After 6 months in the high dose group nearly doubled clearance half time compared to control. After 18 months this effect was not detectable anymore. Examination of blood and urine: Hemoglobin adducts and MDA urine concentrations were found in all MDI groups after 3 and 12 months exposure. A steady-state was observed after 3 months exposure.

Conclusion of the authors

In a long-term inhalation study over a maximum of 24 months including satellite groups with 3, 12, and 20-month exposure, the chronic toxicity and carcinogenicity of monomeric methylene diphenyl diisocyanate (MDI) were investigated. Female Wistar rats were exposed in 6 m^3 inhalation chambers for 17 hours/day, 5 days/week to 0.23, 0.70 and 2.05 mg/m^3 MDI in aerosol form, a control group was kept in clean air. Essentially, 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, an intermediately retarded lung clearance in the high dose group as well as dose-dependent interstitial and peribronchiolar fibrosis, alveolar bronchiolisations and a proliferation of the alveolar

epithelium, which was classified as preneoplastic, as well as a bronchiolo-alveolar adenoma were ascertained. The LOAEC for the female rat was 0.23 mg/m^3 after long-term inhalation of 4,4'-MDI aerosols.

1.2 Skin sensitisation

1.2.1 **Animal data for m-TMXDI**

1.2.1.1 Skin sensitisation study in guinea pigs (BRC, 1981)

BRC (1981): Dermal sensitisation study of compound number 11583B15 and isophorone diisocyanate. Report no. 81-149, date: 1981-10-22. Biosphere Research Centre. Cytec Industries, unpublished

The text below is reproduced from the registrant's summary in the technical dossier, with slight editorial modifications by the DS. For a critical evaluation of the results by the DS, the reader is referred to the main part of this dossier.

Test material

m-TMXDI, analytical purity: 91.58 %.

Test animals

Hartley guinea pigs (sex not specified). Source: Elm Hill Breeding Laboratories, 71 Elm Street, Chelmsford, Massachusetts 01824, USA. Weight at study initiation: 327-498 g. Housing: Individually housed in stainless steel cages with wire mesh floors. Diet (e.g. ad libitum): Purina Guinea Pig Chow, ad libitum. Water: Filtered tap water, ad libitum. Acclimation period: 7 d.

Methods

Range-finding tests

Five animals each were exposed to 25 µL of molar dilutions (0, 0.10, 0.05, 0.025, 0.0125, 0.00625 %) of either the test or positive control article in olive oil. Route: Epicutaneous; no patch was applied.

Main study

(a) Induction exposure:

No. of exposures: Single. Control group: Yes, olive oil (vehicle control) and isophorone diisocyanate (IPDI, positive control). Site: Flank to trunk along both sides of each animal. Frequency of applications: Once. Duration: 5 d. Concentrations: 0.36 molar concentration.

(b) Challenge exposure:

No. of exposures: Single. Day(s) of challenge: 9 d. Control group: Yes. Site: Applied to untreated site, flank to trunk along both sides of each animal. Concentrations: 25 µL of 0, 0.10, 0.05, 0.025, 0.0125 and 0.00625 % molar concentration. Evaluation (hr after challenge): 28 and 48 h.

(c) Other:

Re-challenge Phase: 9 d after the initial challenge. Procedure: Same as challenge. Challenge controls: Not applicable. Positive control substance(s): Isophorone diisocyanate (IPDI).

Results and Discussion

Positive control results

Primary skin irritation phase: At 24 h one male exhibited a grade 1 erythema at the dose levels of 0.1 %; one female exhibited a grade 2 erythema at 0.1 and 0.05 %, and a grade 1 erythema with 0, 0.025, 0.0125 and 0.00625 % (olive oil only). By 48 h, both grade 2 erythemas had decreased to grade 1 and the site treated with olive oil returned to normal. All other test sites appeared normal.

Induction phase: Exhibited grades of 1, 2 and 3 for erythema and no oedema at 24-hour interval. Scores had decreased slightly but were considered comparable at the 48-h interval.

Challenge phase: Mean skin irritation scores were higher at challenge than at the skin irritation phase. Re-challenge phase: Mean skin irritation scores were less or comparable at re-challenge than at the skin irritation phase.

Results with test material

Table 11: Test results for m-TMXDI in a skin sensitisation test in guinea pigs (BRC, 1981)

Reading	Hours after challenge	Dose levels in %	% animals with reactions (n = 10)
1st	24	0.1, 0.05	100
	24	0.025	70
	24	0.0125	90
	24	0.00625	50
2nd	48	0.1, 0.05, 0.025, 0.0125	100
	48	0.00625	70
Re-challenge	24	0.1, 0.05, 0.025, 0.0125, 0.00625	0
	48	0.1, 0.05, 0.025, 0.0125, 0.00625	0

Table 12: Mean skin irritation scores, reproduced from the summary of (BRC, 1981), as presented in the registration dossier

Primary Skin Irritation Phase:												
Concentration	0.1		0.05		0.025		0.0125		0.00625		0.0*	
	Er	Ed	Er	Ed	Er	Ed	Er	Ed	Er	Ed	Er	Ed
IPDI (24 h)	0.6	0.0	0.4	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0
IPDI (48 h)	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.2	0.0	0.0	0.0
11583B15 (24 h)	0.8	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11583B15 (48 h)	0.4	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Challenge Phase:												
IPDI (24 h)	2.7	0.5	2.1	0.0	1.5	0.0	1.1	0.0	0.9	0.0	0.0	0.0
IPDI (48 h)	1.9	0.0	1.9	0.0	1.7	0.0	1.2	0.0	0.9	0.0	0.0	0.0
11583B15 (24 h)	2.3	0.2	2.1	0.2	0.7	0.0	1.1	0.0	0.5	0.0	0.0	0.0
11583B15 (48 h)	2.1	0.0	2.0	0.0	1.0	0.0	1.2	0.0	0.8	0.0	0.2	0.0
Rechallenge Phase:												
A-IPDI (24 h)	0.9	0.0	0.8	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
A-IPDI (48 h)	0.7	0.0	0.6	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B-IPDI (24 h)	0.5	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B-IPDI (48 h)	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11583B15 (24 h)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11583B15 (48 h)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

11583B15 = m-TMXDI; A: Animals treated with IPDI during induction; B: Animals treated with m-TMXDI during induction; * Vehicle (olive oil) only; Er: Erythema; Ed: Oedema

Applicant's summary and conclusion

Under the test conditions, the test material was considered to be a contact sensitiser in guinea pigs (BRC, 1981).

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